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**MEDICAL RESEARCH
COUNCIL**

**T.N.T. Poisoning and the Fate of T.N.T.
in the Animal Body**



LONDON

PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE

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INTRODUCTION

THE occurrence during the War of serious morbidity and mortality, associated with a definite syndrome of symptoms, among munition workers who came into contact with the high explosive trinitrotoluene (T.N.T.) threatened at one time to produce a serious curtailment in the output of munitions. The earlier history of the outbreak, and of the measures set on foot to deal with it, is given in the first memorandum of the following report by Dr. W. J. O'Donovan, who relates how, when the main practical problem had been solved with a large measure of success, the Medical Research Committee, at the request of the Ministry of Munitions, appointed a scientific committee, whose function should be to gather such information, obtained in the course of the general investigation, as seemed likely to have permanent scientific value, and to promote such further investigations as would establish knowledge of the poisonous action of T.N.T. on a firmer scientific basis.

The following report, in addition to the general historical and clinical account by Dr. O'Donovan, includes papers by Dr. G. A. Wyon on the pharmacology of experimental T.N.T. poisoning; by Mr. Webster and by Dr. Dale on the metabolism and fate of T.N.T. in the animal body; and by Dr. Panton on the pathological changes produced by T.N.T. in animals experimentally poisoned and in the human victims of factory poisoning. These papers complete the series begun by the separate issue of Dr. Benjamin Moore's report (Special Report Series, No. 11). The different memoranda were drawn up by their authors at different dates and to some extent independently of one another. They will be found at a few points to overlap, and occasional redundancies and minor discrepancies were inevitable in the circumstances. It has been considered desirable to publish the memoranda as they stand, rather than obtain uniformity at the cost of the delay entailed by communicating with now scattered contributors.

Dr. O'Donovan approaches the subject from the historical, technical, administrative, and clinical aspects, and his memorandum contains an account of the rise and fall of T.N.T. poisoning. He describes how collaboration between research workers, administrators, medical men, and engineers, established such a grip upon the position, that, while in the twelve months from August 1, 1916, to July 31, 1917, there were 238 cases of toxic jaundice with 75 deaths, there were during 1918 only 34 cases with 10 deaths, even though in the last year of the war the total number exposed to risk had largely increased. This part of the report is of interest, not only historically, but as an example of combined action for a common end. Dr. O'Donovan discusses the possibility of an unknown factor, whether due to some intercurrent infection, or to some peculiar lowering of resistance, playing a necessary part in determining toxic jaundice among those exposed to T.N.T. One case quoted wherein toxic

jaundice seems to have been precipitated by traumatism seven months after exposure to T.N.T., is reminiscent of the part sometimes played by traumatism in determining an attack of pneumonia.

Dr. Wyon's memorandum gives clear evidence of the difficulty experienced in producing, in experimental animals, a type of T.N.T. poisoning comparable to that seen in man, and a similar experience is recorded in Dr. Panton's first paper. Interesting conclusions emerge nevertheless from Dr. Wyon's work. It will be noted, for example, that the cat, which shows very much greater sensitiveness to the poisonous action of T.N.T. than the rabbit, does not excrete in its urine any of the chromogenic substance which appears in the urine of the rabbit, monkey, or man absorbing T.N.T., and which has been regarded as the characteristic end-product of the substance, and as an indication of danger when detected in the urine of factory workers. Suggestive in this connexion is the observation recorded by Dr. O'Donovan, that patients suffering from toxic jaundice, due to T.N.T., do not show the colour reaction in the urine, even when a dose of T.N.T., sufficient to produce a clear reaction in the normal person, is rubbed into the skin. From considerations of a quite different kind, based on Mr. Tutin's quantitative data as to proportion of T.N.T. administered to rabbits which is excreted as the Webster chromogen, it will be seen that Dr. Dale, again, arrives at the conclusion that the chromogen represents probably the line of safest metabolism and excretion of T.N.T., and that its *absence* from the urine of a patient known to be absorbing T.N.T. may be the really ominous sign. It is interesting to note this convergence of the different and independent lines of clinical, pharmacological, and biochemical evidence to the same conclusion.

On a point of detail, namely, the exact chemical nature of the chromogen, a discrepancy may be detected between the statement of Dr. Wyon on the one hand, and those of Mr. Webster (its discoverer) and Dr. Dale on the other. Dr. Wyon's statement, and the view he based on it as to the significance of the chromogen in the poisoning process, seemed to be justified by the evidence available when he wrote his memorandum; but some results already obtained by Mr. Webster, and their confirmation by the very careful work of Mr. Tutin, made it necessary to abandon this conception in favour of that which is given by Mr. Webster and by Dr. Dale.

There are several other points on which the different authors are led to similar conclusions. There is, for example, a general consensus in favour of the view that the toxic results were due to the T.N.T. (*a.* trinitrotoluene) itself, and not to any more toxic and variable impurities. On the other hand, the irregularity in the incidence of toxic jaundice, its tendency to appear in factories in outbreaks having the form of small epidemics, and the practically complete failure to reproduce comparable liver changes by the heaviest dosage in experimental animals, lead Dr. O'Donovan, Dr. Wyon, and Dr. Panton, each from his own point of view, to suggest the presence of some other, unknown factor in the production of the liver atrophy. One suggestion considered, that of an infection, harmless or trivial in its effect on the liver of the healthy man, but capable of initiating widespread degenerative changes in the liver weakened by prolonged

exposure to T.N.T., has already appeared in speculations on the causation of the outbreaks of liver atrophy, in many ways so similar, which have occurred among patients heavily dosed with Salvarsan.

The other type of fatal poisoning by T.N.T., the aplastic anaemia with which Dr. Panton's memoranda deals fully, proved more accessible to study by animal experiment. It was a much rarer accident in man than toxic jaundice, and sporadic in its distribution. No suggestion is offered for its appearance in a few cases out of many thousands exposed to T.N.T., other than individual susceptibility.

There are many questions concerning the poisonous action of T.N.T. which these reports leave unsolved. Further progress towards their solution by laboratory experiment does not at present seem probable, and with the termination of the war, opportunities for studying its effects on large numbers of human subjects have fortunately disappeared. Further light on it, if any, will probably come from the study of allied conditions produced by other substances; and, similarly, these studies on poisoning by T.N.T. may be found to have a value, independent of the practical object with which they were undertaken, for the elucidation of pathologically similar conditions produced by a much wider class of poisons.

MEDICAL RESEARCH COUNCIL,
15 Buckingham Street,
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5 November 1920.

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I. T.N.T. SICKNESS AND TOXIC JAUNDICE

BY W. J. O'DONOVAN, O.B.E., M.D., M.R.C.P.

(Dermatological Department, London Hospital)

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(1) Historical.

LONG before the outbreak of war in August 1914, the *manufacture* of explosives involving the use of trinitrotoluene had been recognized as a dangerous process, and, as such, its use was safeguarded by inclusion in Home Office Regulations dated October 3, 1908 ; the substance itself, with which mines had been filled by the Explosives Loading Company, Faversham, for years, had borne a most innocent reputation as to its toxicity. Dr. Prosser White, of Wigan, a recognized authority on poisoning by the toluene derivatives, had described T.N.T. as an innocuous non-toxic substance which had never been known to produce illness. Until the end of 1915 nothing occurred to shake this prevailing belief. Toxic jaundice was added to the schedule of diseases that have to be notified to the Chief Inspector of Factories by a Home Office Order of November 1915, but this was because 70 cases of toxic jaundice with 12 deaths had occurred in various aeroplane factories from the use of tetrachlorethane, a constituent of the dope or varnish applied to aeroplane wings.

The *first death* attributed to T.N.T. occurred in the manufacture of this explosive in February 1915. A man, J. F. B., aged 34, had been employed from October 26, 1914, until February 9, 1915, as a labourer in a T.N.T. manufacturing plant. On the latter date he was suspended by the certifying surgeon for jaundice.

He fell ill on February 11 and died on February 23, 1915. Dr. Legge of the Home Office attended the post-mortem and inquest.

The post-mortem showed grey hepatization of lung in which pneumococci were demonstrated. The liver was normal in size and showed multilobular cirrhosis. It is very dubious whether this case should be properly recorded as one due to T.N.T. poisoning in which acute atrophy of the liver is the outstanding feature, and hence the following case should be considered the first authentic case of liver atrophy due to T.N.T.

On July 9, 1915, Dr. Edgar Collis, after visiting a factory in Kent, travelled on the back of a motor bicycle to Whitstable to see a patient, Rn., under the care of Dr. Rupert Smith, who had connected his patient's illness with the 'inhalation' of trinitrotoluene, and who gave the following history :

Age 19, previously an oyster dredger ; employed filling shells and mines with trinitrotoluene for twelve weeks. Left work a month previously suffering from severe vomiting and inability to take food. He said his bowels only acted after taking salts, and that the motions were clay coloured, also that the urine was deep brown. On examination, the conjunctivae and whole surface of the body were deeply jaundiced but his general condition was not serious. Except for some depression his aspect was normal, pulse 72, lips a good colour. There was no tenderness over the liver, but Dr. Collis, on examination, thought the liver was reduced in size. The patient recovered.

The first undoubted death due to T.N.T. was a man A. W., aged 44, who worked on shell filling at Woolwich Arsenal from May 1915 to July 30, 1915, and died on August 17, 1915, in Guy's Hospital under Dr. Herbert French. The post-mortem showed the atrophic liver changes due to T.N.T., with which we have since become familiar.

In February 1916 no cases of toxic jaundice were reported, but in March, four cases, with three deaths, occurred, and Dr. Legge of the Home Office, realizing that this might become a growing menace, drew up and distributed a small book on the subject for the instruction of medical officers in the factories concerned in the handling of T.N.T. Although up to June 1916 no cases had occurred in any of the newly organized national filling factories, Dr. Legge pointed out to the Ministry the serious consequences to be expected from the handling of T.N.T. in largely increasing quantities, and Dr. W. J. O'Donovan was attached by Sir Eric Geddes, then Deputy Director of Gun Ammunition Filling, to his division, so that the work of the factory medical officers might be organized on proper lines, and the clinical and preventive experience of the different factories might be co-ordinated.

Meanwhile, the number of cases reported to the Home Office began to increase. In July of 1916 there were 16 cases and five deaths, seven of which cases, including two deaths, occurred in the recently established national filling factories. On August 2, 1916, Dr. Addison called a meeting of the representatives of all departments concerned in the use of T.N.T. and from this may be said to date the strenuous efforts, medical, engineering, and administrative, made to put a stop to this growing menace.

Before this date, precautionary measures had been considered by the Health of Munition Workers Committee, and the following

seven cardinal points were set out and may be said to have served as a basis for the elaboration of later regulations :

1. Cleanliness of premises and person.
2. Alternation of employment, by means of which workers would be alternately on and off handling T.N.T.
3. Adoption of proper costumes, particularly for women.
4. The necessity for adequate washing accommodation.
5. Adequate canteen arrangements.
6. Efficient ventilation.
7. Adequate medical supervision.

At the end of September 1916 the position of T.N.T. illness had become much more acute, the reported cases of toxic jaundice rising to 57 with 16 deaths during the months of August and September; moreover a tendency to decline work in factories where T.N.T. was being handled began to manifest itself among the workers. To cope with this latter menace, which from its effects would be so dangerous to the country, the Minister of Munitions, Mr. Montagu, appointed at the end of October 1916 an Advisory Committee under the chairmanship of Sir (then Dr.) W. M. Fletcher with Mr. Gray Barnett as secretary. This committee met almost daily for a short time. Recommendations were issued to secure clean working and good ventilation and the provision of suitable costumes. A memorandum was also issued setting out in detail the functions of factory medical officers. (Appendix p. 28.)

The existing powers of the Ministry of Munitions to enforce regulations were gone into carefully, and on December 22, 1916, Section 35 A.A. was added to the Defence of the Realm Regulations by an Order in Council which gave the Admiralty, the Army Council, and the Ministry of Munitions powers to enforce in any factory any regulation considered necessary in the interests of health.

On January 6, 1917, draft regulations prepared by a sub-committee were finally approved by the T.N.T. Advisory Committee and submitted to the Minister, Dr. Addison, on their behalf by the chairman with an explanatory letter of January 10, 1917. These rules in an amended form were finally issued on March 30, 1917, with a covering memorandum originally drawn up by the T.N.T. Committee.

Feeling that the main work of the T.N.T. Committee had ceased with the issue of these regulations, the Minister of Munitions, now Mr. Churchill, decided that the functions of the T.N.T. Committee lay now in the field of scientific advice, and a T.N.T. Advisory Committee, consisting of Sir Walter Fletcher, Dr. Collis, Dr. Dale, Dr. Legge, Dr. Moore, Dr. O'Donovan, and Dr. Panton, was constituted for the initiation or direction of further research work and its application to the practical problems of the Ministry.

(2) Technical.

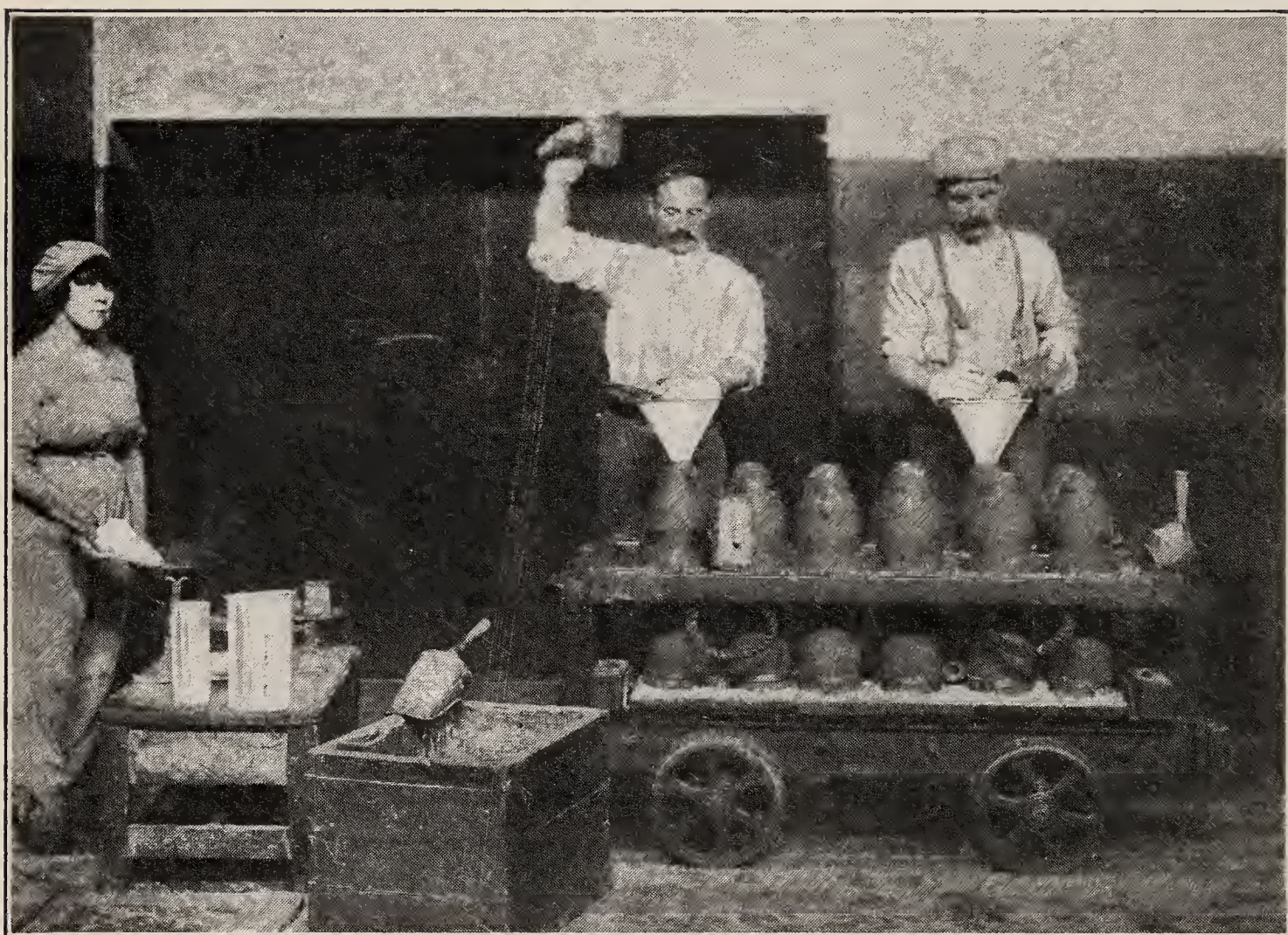
An explosive is a substance which on detonation liberates a large volume of gas with great rapidity. The two factors of power and violence are both essential. It is well recognized that petrol in burning gives off more power than is liberated by the detonation of a high

explosive in the ratio of something like 10 to 1. But burning petrol is a comparatively slow process and hence the effects of the burning of petrol and the detonation of an explosive can hardly be compared. The power of an explosive is commonly decided by making a hole in a lead block, plugging the hole with a steel bung, and then firing the explosive by means of a fuse. Under these conditions the lead block will swell out and water poured into the cavity will give a rough indication of the amount of gas liberated. The violence of an explosive is measured by the rate at which the explosion spreads in a column of material, technically spoken of as the velocity of detonation. The power of T.N.T. is about 95 per cent. of that of picric acid. The velocity of its detonation is about 7,000 metres a second, that is to say a wave of detonation will run through a column of T.N.T. at 7,000 metres a second as compared with 7,300 for picric acid.

T.N.T. is relatively a scarce commodity. Ammonium nitrate can be manufactured in very large quantities, and therefore to economize T.N.T. and to make the fullest possible use of ammonium nitrate, dilution of T.N.T. by ammonium nitrate was a matter of early trial and experiment. The earliest mixture was roughly half of T.N.T. and half of ammonium nitrate. This was sufficiently fluid when hot to be poured under favourable conditions, but it was found to be a little too thick for factory operations, so that gradually the mixture became one of 40 per cent. ammonium nitrate and 60 per cent. of T.N.T. or 40/60 AMATOL, but in the latter months of the war another mixture was introduced consisting of four parts of ammonium nitrate to one part of T.N.T., 80/20 amatol. As regards power, amatol is more efficient than either picric acid or T.N.T. alone; taking picric acid as a standard and calling it 100, the power of 40/60 amatol is 116 and 80/20 amatol is 127, and as regards crater effects an explosive containing a large proportion of ammonium nitrate proved better than an explosive consisting of pure T.N.T.

A great merit, however, of a high explosive is its insensitivity. Amatol can be filled into a shell in a manner that would be dangerous with black powder: the firing of a charge of cordite behind a shell is sufficient to give its contents a severe shock, and if the shell were filled with a sensitive explosive there would be a very real risk that the shock of the charge would cause immediate detonation of the shell contents. In firing, too, against concrete dug-outs or armoured shelters it is important that the shell shall not detonate upon impact but shall at first attain some measure of penetration. Experience has shown that shell cases have been knocked in by the detonation of other shells and yet the amatol contents inside have not been detonated. The importance of this factor to ammunition dumps cannot of course be over-rated.

The manner in which insensitive explosives are detonated is by means of a series of relays. The detonation is started by mercury fulminate which is very sensitive and fairly violent. This sets off an intermittent explosive called tetryl. The third relay or stage is crystals of T.N.T. Between the crystals of T.N.T. and the amatol in the shell is some cast T.N.T.



This illustration shows the hand-stemming of T.N.T. into a shell.

A girl in working costume on the left is weighing a charge of amatol which is emptied from the scale-pan into a metal receiver. The man on the right is emptying a charge into a shell and the workmen in the middle is hammering down the powder with a wooden rod and mallet. The exposure of the worker's hands and soiling of the floor is evident.

It will be seen from these remarks that T.N.T. may be used as an intermediate factor in the detonation of an amatol-filled shell or it may be used as amatol for the filling of grenades, howitzer bombs, and shells of all sizes. These latter terms call for no explanation.

Exploder Bags.

These consisted of cylindrical batiste bags holding about 19 drs. weight of T.N.T. They were originally filled by hand through funnels, then by horizontal or vertical machines in the nature of sausage fillers with automatic arrangements for starting and stopping when the required weight was put into the bags. After filling the bags were tied with silk thread and the superfluous silk and batiste were cut away.

In the latest form a paper carton replaced the batiste bag, the open end of which was closed after filling by a cardboard disk. This method could be worked to a greater degree of accuracy than was possible with the batiste bags; it reduced the amount of manhandling of T.N.T., and the cost of filling these cartons was considerably less than the filling of batiste bags by machinery. Most recently the T.N.T. was filled by automatic machinery directly into the metal exploder container without any bag or carton at all. This most rational way was the last thought of. All filled shells had a cavity left at their summit into which the exploder container was screwed, and the direct filling of the charge into this cavity was a noteworthy explosive and medical improvement in the handling of T.N.T.

Methods of Shell Filling.

The early method of filling molten amatol through spigots into shells involved the labour of scores of workers removing caked amatol spilt on to the outside of shells and on to trucks, floors, and runways. In one large factory, on each of two shifts in the melt house there were between 50 and 60 women workers almost wholly employed in cleaning up owing to the spilling of amatol. Many workers engaged in trucking and other work, apart from the actual filling of the shell, came into contact with T.N.T. Dr. Moore noted that the heaviest incidence of both fatal and non-fatal cases of jaundice was found at one factory among the cleaners and truckers and not among the melters and pourers.

In filling shells by the press process, T.N.T. and ammonium nitrate had to be separately melted and then mixed. The amatol mixture was conveyed by trucks or automatic conveyors to press houses where the correct charges were weighed by hand and emptied through shell funnels into the waiting shells. These charges were rammed home by means of a process of tamping or stemming; the stemmer poured the powder through a funnel inserted into the shell opening and then hammered down the powder by striking the upper end of a wooden rod with a wooden mallet. At each stroke a fine gust of powder was driven up between the stem and the side of the funnel and another gust between the funnel and the side of the shell. In consequence of this a thick layer of dust fell upon the trucks,

floors, and workers, and so was inevitably distributed out of the precincts of the press house along the runways and into the shell stores.

Screw Filling.

For labour saving reasons in June 1916 technical officers of the Ministry were interesting themselves in mechanical methods of filling exploder bags and shells, but real and speedy progress was made when the need for eliminating hand work for medical reasons was put before the responsible officers of the Ministry of Munitions ; on December 18, 1916, at a meeting of factory managers at Aintree a demonstration of a filling machine in action was given.

Lord Chetwynd first took up the question of shell filling at Chilwell in September of 1916. His initial experiments were carried out with a corkscrew revolving inside a metal tube passed through the neck of a glass bottle and in March of 1917 an extremely satisfactory vertical filling machine with a rising hopper was in use at this factory. But before this date toxic jaundice at this factory had become an almost negligible factor. With this machine 6-inch shells could be filled in 45 seconds. A horizontal machine was the latest type brought into use, superior in some ways to the vertical machine since manipulation is easier in the horizontal position, and on the withdrawal of the filling screw any T.N.T. dropping from the end of the screw falls clear of the shell into a tray below and hence contamination does not spread away from the actual site of filling.

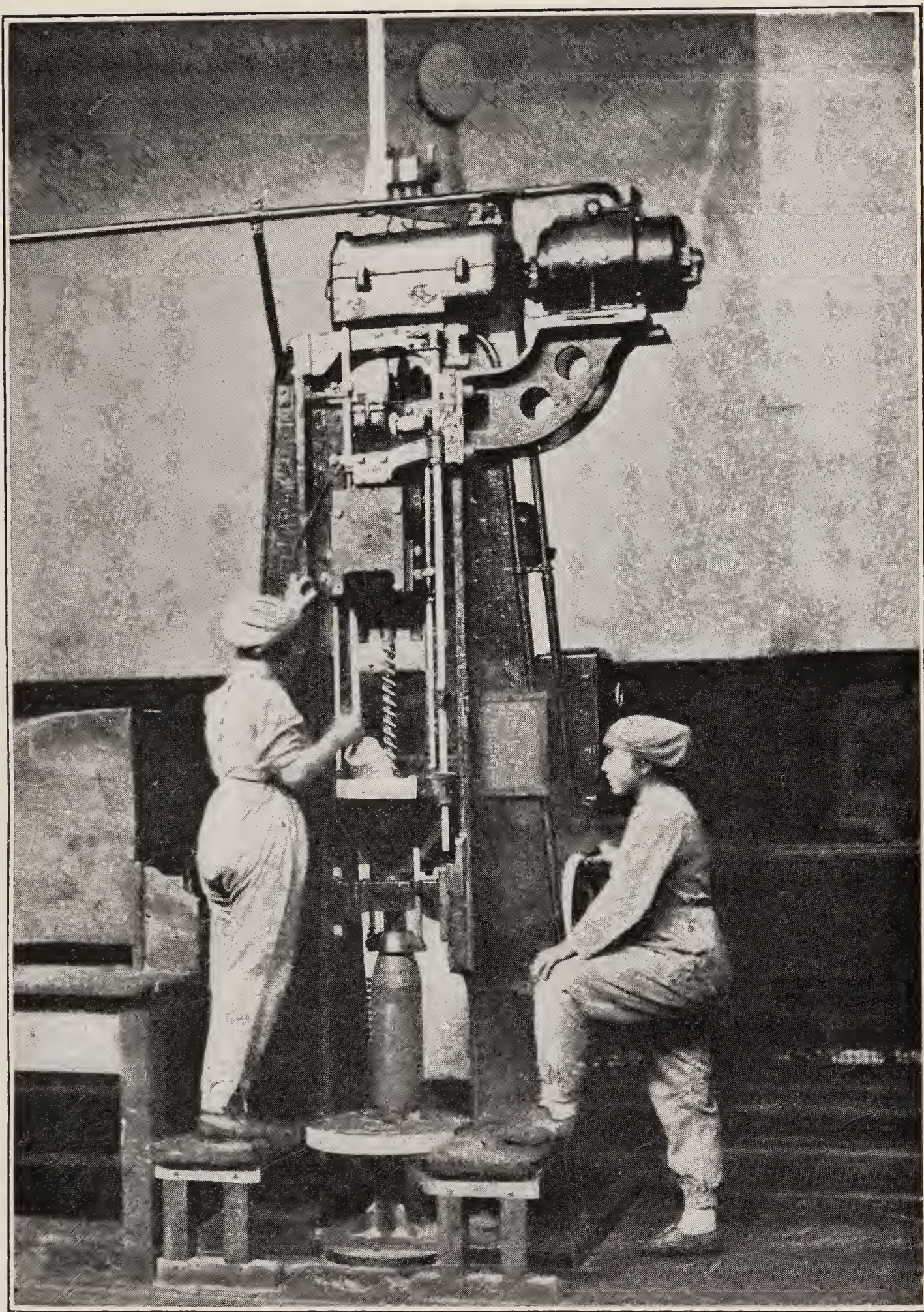
Hand Grenades.

Hand grenades of such patterns as the Mills grenade and the 'Egg' grenade owing to their internal structure were of necessity filled by hand. The actual process consisted of filling a metal cone about 18 inches long with an explosive mixture. Compressed air was then admitted to the base of the cone and its apex was inserted into a filling hole in the side of the grenade. A dozen bangs of the grenade on the work bench distributed the powder evenly in the grenade. In this process the work bench and grenades were thickly dusted with ammonal and deep staining of the workers' hands was common, yet although many thousands of these grenades were filled by hand, dermatitis has been the only ill effect noticed in the process of grenade filling. A partial explanation of this paradox of dirty work and good health can perhaps be found in the low percentage of T.N.T. present in the filling mixtures used, viz. 10 per cent. or less.

(3) Clinical Effects.

The ill effects that T.N.T. workers suffer from can be classified under five main headings :

1. Dermatitis.
2. An early, probably reflex vomiting.
3. Affections of the blood or blood-forming organs.
4. Toxic gastritis.
5. Toxic jaundice.



Chilwell stemming machine in operation ; powder being fed to hopper by the girl on the platform.

(Although by analogy with other benzine derivatives one would have expected cases of peripheral neuritis and of toxic amblyopia among T.N.T. workers no such cases have come before the medical officers of the Ministry.)

DERMATITIS.

In this susceptibility may be said to have played a large part. Most workers were never attacked, whereas a few suffered severely from it even after exposure of slight duration. Most frequently it was met with in the first week of exposure, but very intractable cases were met with in workers who had handled T.N.T. with impunity up to twelve months. The parts most frequently affected were the wrists and ankles and neck, where rubbing with contaminated clothing occurred, but facial and intercrural attacks were not uncommon. The rash usually appeared as a superficial, follicular or widespread erythema, but on the hands a chronic eczema or cheiropompholyx type of eruption were also very common. Generally, T.N.T. skin affections yielded readily to simple treatment, but septic secondary infection and thrombo-phlebitis were occasionally seen. In all forms itching was often intolerable. Yellow staining of the hands and occasionally of the feet of T.N.T. workers was universal. The staining was a deep orange colour produced after very slight contact in those having greasy skins, and was most marked among operatives handling T.N.T. containing a high percentage of impurities. There was no connexion between the prevalence of dermatitis and the amount of T.N.T. sickness, and only in a very few cases have the same patients suffered from dermatitis and constitutional ill effects either together or at subsequent periods.

Powder Holes. The occurrence of ulcers in the skin and mucous membranes due to industrial work has been recognized for some time among chrome workers, workers in fulminate of mercury and other dusty occupations, but it was not until August 1918 that the occurrence of powder holes was observed among T.N.T. workers.

On August 19, I (W. J. O'D.) visited Messrs. Curtis's & Harvey's factory at Faversham, and the T.N.T. workers on that date, in all 49, were carefully examined. Twenty-six of these showed minute ulcers on the fingers attributable in the first place to injury. The fingers showed recent cuts, recent blisters or dried old blisters, some of which were black with hardened blood clots. It was noteworthy that these injuries had not healed in the usual clean fashion customary in engineering and other workshops, but the tendency was for these linear cuts or abrasions of the fingers and thumbs to become shallow round ulcers which at the time of my visit were of four, five, and up to eight days' duration. Of even greater interest was the presence of five cases showing definite 'powder holes' in the webs between the fingers or between the fingers and thumb. Noteworthy features at the visit were the free sweating and deep walnut staining of the workers' hands, and the tremor of the hands when extended. The tremor was attributable to the effects of a recent explosion and was less marked than it had been some days previously. It was striking that none of the workers handling flaked T.N.T. as opposed to those handling amatol powder showed any of these cutaneous

effects, nor did the girls whose work consisted of dipping solid pellets of T.N.T. into molten wax show any such lesions: hence, the hygroscopic property of ammonium nitrate, the major constituent of amatol, must be at least an important factor in the production of the powder holes. A striking feature was the absence of complaint by the workers as to any suffering from these cutaneous lesions; but such anaesthesia is common with powder holes due to industrial processes, and is the reason why they often go untreated for weeks, and on this account perforations have been recorded that reach down to the phalangeal bones.

Such 'powder holes' are to be attributed to the continued attrition of caked amatol and sweat stuck in rolls in the webs of the fingers, and the non-healing and ulceration of small cuts and abrasions is to be put down to the irritant effect of amatol upon undressed cuts.

FUNCTIONAL OR REFLEX GASTRITIS.

Among new workers vomiting in the early morning and occasionally after food during the first fortnight was a fairly frequent complaint; absentees from this cause were extremely rare. Similar complaints were made among workers in fulminate of mercury and in tetryl sheds, and the issue of free milk and a purge was in all cases sufficient to bring about a cure. The explanation of this seems to be largely psychic, inasmuch as after the first two years hardly any sickness of this type was seen by the factory medical officers.

BLOOD CHANGES.

Noticeable pallor of T.N.T. workers in the early days led to a special investigation of this question by Dr. P. N. Panton. The effect of these researches was to narrow down the field of blood changes to a uniformly fatal lesion termed *aplastic anaemia*. In all 15 cases of aplastic anaemia are known to have occurred and all are now dead. Four only had previous toxic jaundice.

APLASTIC ANAEMIA.

This disease may show itself as gradually increasing debility and breathlessness as found in pernicious anaemia, or the onset may be sudden and haemorrhagic. The haemorrhage may show itself first on the legs, as a general purpura, or as a case of severe uterine bleeding. Too much stress cannot be laid on the necessity for a skilfully conducted post-mortem inasmuch as a fatal uterine haemorrhage has certainly in two cases been ascribed wrongly to abortion and the cutaneous haemorrhages of both septicaemia and leukaemia may be and have been wrongly ascribed to T.N.T. when occurring in T.N.T. workers.

There is no parallelism in the incidence of toxic jaundice and aplastic anaemia. One factory, 'C', with the largest number of cases of toxic jaundice, has had only one case of aplastic anaemia, whereas another factory, 'H', with only a few cases of toxic jaundice, has had four cases of fatal anaemia.

TOXIC GASTRITIS.

This was the outstanding ill-effect experienced in all filling factories. The incidence varied from factory to factory, but appended is the incidence of sickness in the factory which suffered more than any from this disease. The number employed averaged about 6,000 throughout the period. The amount of sickness in percentages on the different processes was as follows :

<i>Week ending.</i> 1916.	<i>Melt filling.</i>		<i>Press filling.</i>		<i>Incorporating mills.</i> Males only.
	Male.	Female.	Male.	Female.	
October 7 . .	4.0	5.9	3.2	2.5	5.3
October 14 . .	7.7	10.0	4.3	5.1	5.7
October 21 . .	11.4	8.7	3.8	7.2	7.4
October 28 . .	4.1	3.1	4.5	5.6	3.0

Pain was the general and often the only complaint. This pain started in the epigastrium or in the lower chest, always local, and not radiating into the back or in any other segmental area, it varied from tolerable discomfort to great severity, was colicky in character, and had no definite relation to meals. Rest relieved the pain. Neither food nor starvation were palliatives. Vomiting rarely coincided with this pain and when it occurred gave no relief. Nausea and aversion to food were concomitant symptoms, and loss of weight went hand in hand with this. Constipation was the rule ; the presence of diarrhoea always made one suspect the case was one of food poisoning rather than a real effect of T.N.T. absorption. On examination these patients showed marked apathy and muscular weakness, their faces were pallid, drawn, and wizened, their sclerotics were dulled, their tongues clean. Highly coloured urine and frequency of micturition were commonly complained of by these patients. In these cases cyanosis of lips and tongue was generally notable. Abdominal examination in a small proportion of cases showed epigastric tenderness, but usually nothing. These symptoms would come on at any time from a fortnight to nine or ten months after employment on T.N.T., and might exist for a week or two before medical help was sought. Two to six weeks might elapse before the patient was again fit for work. Open air and purgatives were proved the best form of treatment. Relapses were not common, but when they did occur were a warning that this particular patient should be kept from any further contact with T.N.T. A noticeable feature among these patients was the undue prolongation of convalescence owing to the fear of return to work at shell filling, and from this cause chronic invalidism at one time loomed heavily in the statistics of factory illness.

TOXIC JAUNDICE.

Up to the last the factory doctors maintained the view that toxic jaundice might appear without symptoms, and the fact that there had to be an elaborate organization for picking these icteric cases out from among their fellow employees while at their work is sufficient evidence that the early onset of jaundice need not be associated with sufficient symptoms to lead the patient to seek medical advice.

It was an early experience that young adults were frequently attacked and when attacked experienced a high mortality. In consequence of this, employment in T.N.T. work below the age of 18 was prohibited. It has been suggested at different times that alcohol, syphilis, adenoids, obesity, and bad feeding, are predisposing causes, but no evidence is available pointing in any of these directions. The youngest case of toxic jaundice reported was a boy aged 14, the eldest a man aged 52.

The *duration of employment* on T.N.T. before the onset of jaundice varied from three days to seventeen months : the greatest incidence of jaundice fell in the third month of employment, but one has to remember in this connexion the instability of the women workers in the filling factories ; the subjoined table by Captain M. Greenwood, 18.3.18, shows very clearly the constitution of a munitions population by length of service.

<i>Length of service.</i>								<i>Number (percentage).</i>	
1 month	.	.	.	.	.	.	.	.	13.33
2 months	.	.	.	.	.	.	.	.	9.66
3 "	.	.	.	.	.	.	.	.	10.00
4 "	.	.	.	.	.	.	.	.	11.61
5 "	.	.	.	.	.	.	.	.	10.04
6 "	.	.	.	.	.	.	.	.	7.46
7 "	.	.	.	.	.	.	.	.	5.89
8 "	.	.	.	.	.	.	.	.	5.67
9 "	.	.	.	.	.	.	.	.	5.87
10 "	.	.	.	.	.	.	.	.	4.06
11 "	.	.	.	.	.	.	.	.	5.59
12 "	and over	.	.	.	.	.	.	.	10.81

SYMPTOMATOLOGY OF TOXIC JAUNDICE.

The early signs and symptoms of toxic jaundice were of paramount importance, since the earlier these cases were removed from their work the better was the prognosis.

Of 30 cases experienced by one medical officer, 15 had premonitions, their priority varying from a day to a month, generally about a week, of dizziness, tiredness, headaches, and an overpowering desire to sleep in the daytime. Dyspepsia, abdominal pain, and frequent vomiting, were present in varying degrees in most of these cases. In the remaining 15 cases, however, *there was no warning*.

The earliest published observations by Dr. A. Livingstone-Learmouth and Dr. Barbara N. Cunningham stated, in the *Lancet* of August 12, 1916 :

‘It is curious that there seems to be, as it were, no constant relationship between the severity of the symptoms complained of and the ultimate issue of the case. A worker with severe gastric derangement when taken off T.N.T. may completely recover in a short time, whereas slight loss of appetite and lassitude may (although the worker is removed from all contact with the powder) prove the gateway to serious illness and physical disaster. A patient, too, may be jaundiced for some time, and present no more symptoms than are seen in a simple catarrhal jaundice until a week or so from the end, so far as our limited experience of the serious cases enables us to judge.’

Workers deeply jaundiced, such as were met with in the early days of intensive output, were picked out by the medical officers, and these workers often resented strongly any interference with their continuous employment.

In many cases an absolute diagnosis of jaundice was difficult in its early stages ; so many workers have a transient loss of the whiteness of their sclerotics from ill-health, constipation, and other causes, and here a laboratory test has been used with great advantage. A little blood of a suspected worker is drawn into a collecting tube and allowed to coagulate. The serum above the clot is occasionally a vivid yellow colour, and it was the practice of Dr. Castellain at three large filling factories in succession to remove from T.N.T. contact any one whose serum gave such a positive colour test. The factory nurses early became adepts at performing this little routine investigation, and only positive cases were brought to the notice of the factory medical officers.

Vomiting. This symptom in cases of toxic jaundice was often severe, recurring with each attempt to take solid food ; it was rarely absent when jaundice was at all severe. The appetite was generally very good, except when patients were prostrated in a cholaemic state. Constipation was the common accompaniment and the stools were generally white and hard, though natural coloured stools were passed in some cases and viscid bile in fair quantity was often found in the gall bladder at post-mortem examinations.

Abdominal Pain when present was in the liver region and marked tenderness on pressure over the liver area was occasionally elicited.

Liver Dullness. Although this is a somewhat variable area depending on the obesity of the worker, the degree of emphysema and flatulence existing in a particular case, nevertheless many definite observations of diminution of liver dullness have been made by different practitioners and a complete absence of liver dullness before death has been twice noted. In early and slight cases a slight increase of the liver dullness has been noted, but it is impossible to say that in such slight non-fatal cases there were present in fact the typical T.N.T. atrophic changes. Dr. Barbara Crawford has reported a case in which in five weeks the liver dullness from being less than one inch increased to two and a half inches (*B.M.J.*, April 20, 1918).

This regeneration after extensive reduction is indeed noteworthy.

In two cases of toxic jaundice X-rayed, one by Dr. Finzi and one by Dr. Gilbert Scott, shrinkage of liver was demonstrable on the screen.

Ascites. Although a slight amount of ascitic fluid is not uncommon at the post-mortem of these cases, its presence in any quantity sufficient to be demonstrable clinically has been very rare.

Fatal Case of Ascites.

F. C. W., aged 48. Measles, yes ; scarlet fever, no ; rheumatic fever, no ; pneumonia, no. Never abroad. Labouring work usually. Went to Barnbow N.F.F., July 23, 1918. Passed in as fit. Worked on pressing, machine stemming (80/20 cold), and hot mix (80/20) machine stemming. No alternation of employment.

Well until October 3, 1918. Slight jaundice noticed. Complained then to doctor of dragging in lower chest and upper abdomen which is getting worse.

On examination (October 16, 1918). No vomiting. Slight headache. No delusions. Stools grey. Urine reddish. Sweat not noticed. Appetite gone. Bowels loose. Pruritus troublesome. Feels weak and tired. Intense yellow

sclerotics and skin. Heart rhythm, size, and sounds, natural. Pulse 84, regular. Lungs crepitations at both bases behind. Abdomen distended, flat umbilicus, *shifting* dullness, fluid thrill; oedema of sacrum. Slight oedema of ankles.

Post-mortem by Capt. M. J. Stewart (Leeds Royal Infirmary). Peritoneum contains several pints of clear, deeply bile-stained fluid. Liver shows considerable reduction in size. Weight 39 oz. The surface of the organ presents the typical appearance of T.N.T. atrophy, &c. . . . The larger bile ducts and the vessels of the liver present no naked eye change. The gall bladder contains yellow normal looking bile and its mucous membrane appears normal.

Pulse Rate. The pulse rate varies even in similar cases. Of three cases seen on the same day in Hereford Hospital, October 29, 1918, F. B., severe, pulse 100-60; M. H., slight, pulse 44; and B. E., severe, pulse 42-76.

Pruritus. This symptom may be present in jaundice due to T.N.T. atrophy as in other cases of jaundice, it was often entirely absent.

Pyrexia. As a rule patients with toxic jaundice show a normal temperature chart. An irregular pyrexia of 102° Fah. is not uncommon. High temperature by no means indicates a fatal issue.

Case of T.N.T. Liver Atrophy with High Fever, recovery. (Chart A).

F. T., aged 19, single. Temp. 104.4, recovery. After one month's filling shells with molten T.N.T. the patient felt unwell with pain in the stomach, vomiting, slight diarrhoea, and jaundice. Admitted to the Nottingham General Hospital October 16, 1916. Abdomen; no rigidity, no tenderness, no enlargement of organs. Urine 28 oz. per diem, bile present.

The succeeding temperatures were normal or subnormal until the discharge, 27.11.16.

Case of T.N.T. Liver Atrophy, Delirium, and slight Pyrexia, recovery. (Chart B).

T. M., male, aged 22. Worked in T.N.T. from January 25 to April 5, 1917, 5.4.17 complained of faintness: 16.5.17 saw Dr. C., epigastric pain, conjunctival and general icterus noted, admitted to hospital: 2.6.17 delirious, incontinence of urine and faeces, greatly emaciated. Liver dullness 1½ inches in nipple line: 22.6.17 liver dullness barely 1 inch: 27.7.17 discharged, no symptoms, no jaundice, liver dullness 2½ inches: 6.8.17 occasional pain over liver: 27.8.17 liver dullness normal, discharged from factory at own request to take up work in a pit.

Mental Symptoms. In cases of established jaundice the mentality of the patient is usually unclouded and hopeful and patients in bed in hospital usually express a desire to eat more than is given them and to be up and about. This optimism has been aptly likened by Dr. J. A. P. Barnes to *spes phthisica*.

This want of comprehension of the gravity of their illness has been compared by many to that found in cases of puerperal septicaemia, and has made the retention of these patients in hospital a matter of difficulty. Unconsciousness or a quiet delirium may supervene quite suddenly in patients jaundiced and feeling well, but such cases may recover.

DIFFERENTIAL DIAGNOSIS OF TOXIC JAUNDICE.

The diagnosis of toxic jaundice has always been a matter of difficulty that increased with the experience of factory medical officers.

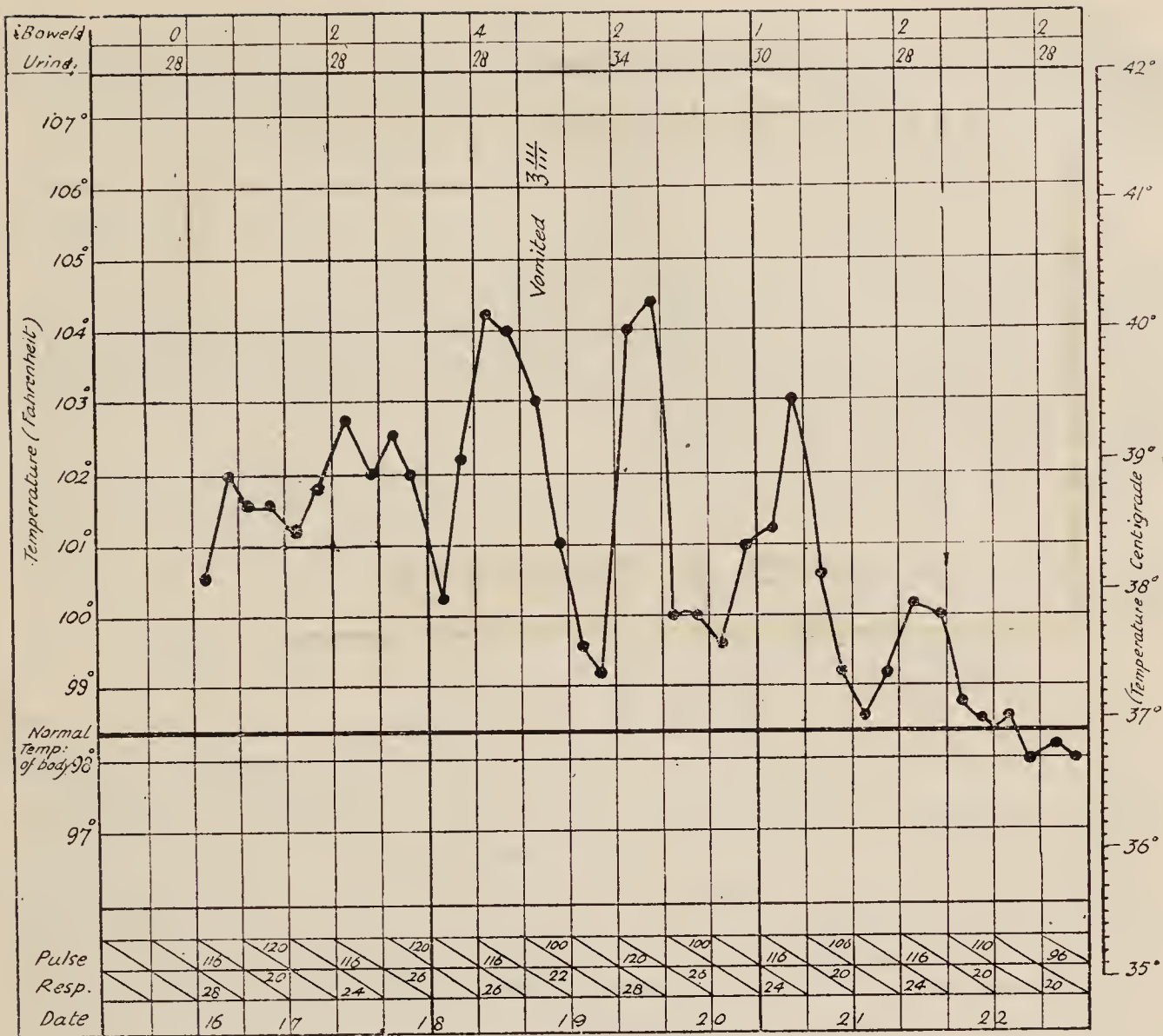


CHART A

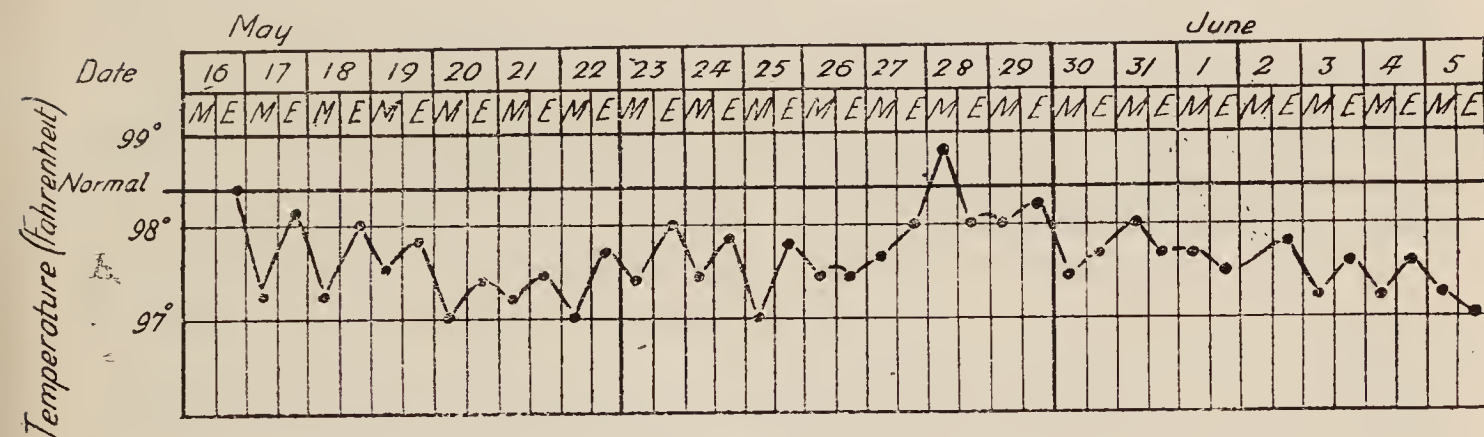


CHART B

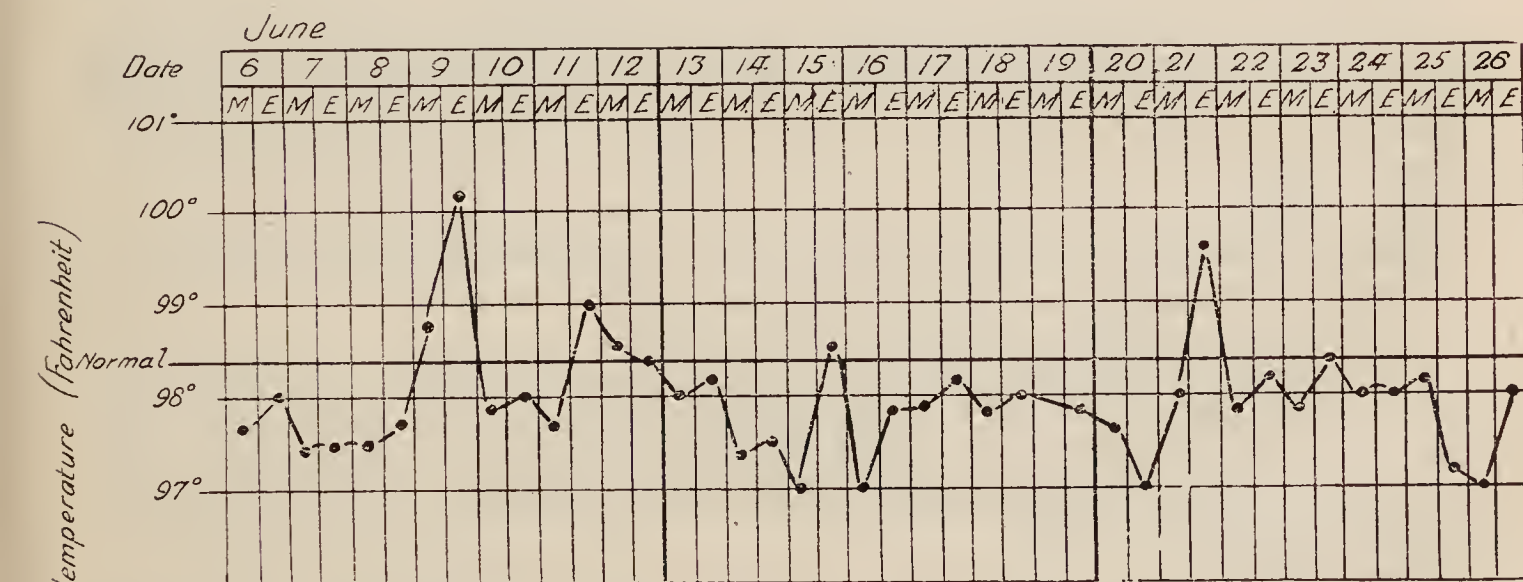


CHART B (continued)

When cases are picked out with slight icterus from among the general body of the workers these cases are symptomless and apyrexial and the factory doctors uniformly stated their inability to distinguish these cases from the catarrhal jaundice of ordinary experience.

This difficulty of diagnosis has been previously referred to by Dr. H. Morley Fletcher of St. Bartholomew's Hospital (*Proceedings of the Royal Society of Medicine*, January 1917).

In severe cases of rapidly increasing jaundice, generally the diagnosis of liver atrophy will be correct, and the exclusion of the rarer causes of grave jaundice among young and middle aged munition workers is not as a rule difficult.

Cases of secondary carcinoma of the liver, familial acholuric jaundice, of gall stones, and large cirrhotic livers, have been met with; but their exclusion, as being cases not due to T.N.T., is not difficult after full investigation.

A noteworthy feature in cases of toxic jaundice has been the absence of Webster's reaction in the urine of these patients, and Dr. Barbara Crawford carried out the following experiment to see whether or no the inability to excrete the T.N.T. derivative giving Webster's reaction might be used with safety for diagnostic purposes:

'From a series of several hundred examinations of the urine my experience has been that T.N.T. is normally present (Webster's test) when a worker is in contact with this substance, and that instead of this being a danger signal, as has been held, it is on the contrary normal and quite compatible with good health; it is not found in the urine of patients suffering from toxic jaundice. I have recently confirmed this at the suggestion of Dr. O'Donovan, by rubbing a small quantity of an ointment containing $12\frac{1}{2}$ per cent. of T.N.T. into the skin of a healthy adult as control. The urines were tested for the next 48 hours with the result that no T.N.T. was detected in the toxic jaundice patient's urine, while it appeared in that of the control after a few hours.' April 1918.

A diminution in liver dullness that progresses while under observation might be regarded as definitely diagnostic in a T.N.T. worker of toxic jaundice.

A diagnosis after the event in these cases is often difficult. A patient who has given a history of jaundice after work on T.N.T. associated with prostration, white stools, and pain in the liver area, may later show a fairly clear picture of cholelithiasis. It is to be remembered, however, that gall stones have been found post-mortem in cases of T.N.T. workers dying of liver atrophy.

It may be held that the presence of gall stones may predispose a worker to an attack of toxic jaundice, and the present certain diagnosis of gall stones does not negative a previous jaundice of toxic origin.

Illustrative Case of Gall Stones and Jaundice in T.N.T. Worker.

Mrs. L. Under Dr. Alexander of Faversham.

Married. One child. Husband alive and well.

First went to work at a factory in Faversham, May 1917. Was employed as an examiner gauging T.N.T. pellets on alternative fortnights. This work stained her hands yellow. Alternate fortnights were spent on non-T.N.T. work. She last left the factory about beginning of November 1917. She was jaundiced about November 23, 1917.

Onset of Illness:

Symptoms: Headache, nausea, malaise. Attacks of pain in R. hypochondrium, which came on at any time day or night, severe, radiated up to right

shoulder and bore no relation to meals. She shivered and sweated in attacks of pain. No vomiting. Her friends noticed her jaundice concurrently with onset of symptoms about a week before she saw a doctor. She has improved since Xmas 1917, though when up a fortnight ago her feet swelled. She still has some pain in R. hypochondrium.

On Examination:

A well built, well covered, fresh complexioned brunette. No cyanosis. No rash. No stains on skin. Tongue clean. Glands not enlarged. Heart: natural rhythm, rate and sounds. Lungs resonant all over, good air entry. Abdomen not distended, no ascites, moves well. A band of hyperesthesia (10th dorsal segment) on right side of trunk from vertebral column to umbilicus. Liver not enlarged, not diminished to percussion, not tender on pressure. Gall bladder: a tumour about an inch across palpable in G.B. area, descends with inspiration, very tender on pressure. Urine has contained bile off and on since first attack.

Diagnosis (11.2.18.):

Gall stones with attacks of colic and some inflammation of gall bladder. The original diagnosis of Toxic Jaundice in 1917 is open to question.

Fatal Case of T.N.T. Liver Atrophy with Single Cholesterin Gall stone.

Case of J. S., aged 49. Morecambe.

Worked in T.N.T. from January 1917 to July 13, 1917.

It is uncertain whether he was medically examined before commencing, all records having been destroyed in an explosion.

July 13, 1917. Noticed to be slightly jaundiced and sent to Lancaster Infirmary.

July 29, 1917. House surgeon could not detect any jaundice.

August 11, 1917. Discharged from infirmary.

October 16, 1917. Death.

Post-mortem, October 17, 1917. Liver 34 oz. Many adhesions, subacute yellow atrophy.

Oval cholesterin gall stone (4×2.5 cm.) present in gall bladder, duct impermeable.

The gall stone was very light in weight and almost floated in water. The surface was smooth and light green. On the cut surface was a peripheral zone (0.15 cm. broad) of soft friable cholesterin tinged by bilirubin. Internal to this were radially arranged large crystals of cholesterin: these were clear and glassy except in the centre of the stone where they were rusty.

Fatal Case of T.N.T. Liver Atrophy with Twelve Gall Stones

M. J., aged 22. Single. Morecambe National Filling Factory.

Commenced work January 17, 1917. Stemming eight weeks. At the end of this complained of headache, vomiting, and abdominal pain. Transferred to kitchen cleaning on February 26, 1917.

March 16, 1917, reported and asked to be sent back to powder. Conjunctivae noticed yellow. Kept under observation till March 20, then sent to Lancaster Infirmary, general jaundice.

Died April 27, 1917.

Post-mortem May 2, 1917. No skin petechiae. Liver 23 oz. No recognizable liver pattern, close set grey points of fibrous tissue. Right lobe contains an area 3"×4" of coalesced pin head yellow nodules. Gall bladder, old adhesions to liver and duodenum. No thickening of wall, contained 12 facettled stones. Bile duct patent.

Fatal Case of T.N.T. and Liver Atrophy associated with Hydatid Cyst.

A. F., aged 22, single, Morecambe National Filling Factory.

Previously a weaver.

Medically examined and passed fit February 13, 1917. Stemming February 13-27, 1917. Transit shed till March 6. Stemming and trucking T.N.T. till March 9.

Left factory April 2, 1917, and returned to home in Burnley; was noticed to

be yellow on arrival. She did two days' weaving but became breathless and saw Dr. Scott, who certified T.N.T. poisoning.

Admitted Burnley Infirmary April 28, 1917. Collapsed, persistent vomiting, general jaundice, delirious, no temperature.

Death May 6, 1917. *Post-mortem.* Blue subcutaneous haemorrhage left shin. General icterus. Liver 38 oz. Left lobe very small tongue. On section red tissue without recognizable liver pattern. Discrete or coalesced yellow lobules scattered throughout. On convexity of right lobe and on under surface of right lobe internal to gall bladder are two hydatid cysts, 3 and 4 inches in diameter. Severe fatty degeneration of heart and kidneys.

LATENT PERIOD.

A remarkable feature of both toxic jaundice and aplastic anaemia in a few cases has been the latency of the condition, that is, the length of period that elapsed between absorption of the poison and the development of jaundice or of anaemia.

The first noted case of this latency was a boy C. N., aged 16, who worked in a melt house from May 23 to the end of June 1916. He was discharged for threatening to blow the place up. He worked at his father's farm from the end of June to August 28 and in the middle of this period felt sleepy for three days. On August 28 he saw his doctor for vomiting. On September 1 he was jaundiced and on September 9 he died. A post-mortem two days later showed an 18½ oz. liver with typical changes of acute atrophy and fatty degeneration.

Another case reported by Dr. Legge in the annual report of the Chief Inspector of Factories and Workshops for the year 1917 was a woman who after two months' work in the manufacture of T.N.T. was transferred to another department where she was employed in filling drums with benzol and toluol. Over seven months later she met with an accident, a spanner falling on her head. Toxic jaundice developed one week later and proved fatal in a fortnight.

The longest period of latency known has been published by Professor Glynn where the latent period extended over nine months. The patient was a female aged 20, single, and was employed from January 1917 up to September 1917 in a filling room where eighteen-pounder shells were filled with amatol and T.N.T. blocks. Her duty was to handle the filled shells. During this period her health was good. She remained well until June 5 when she vomited. On June 7 slight jaundice appeared and she died in the Liverpool Royal Infirmary comatose on June 14. *Post-mortem.* Liver weighed 41 oz. Capsule not wrinkled. Surface, yellow ochre, mottled with small sub-capsular haemorrhages; on section very soft and uniformly yellow. Microscopical examination of the liver showed extensive focal necroses affecting about a third of each liver lobule (central portion).

(4) Preventive Measures.

The preventive measures that have been in use were direct and indirect in their action.

Under indirect measures are included the provision of canteens, good meals, the shortening of hours, the facilitating of workers' transit to and from work and the introduction where possible of

breaks to lessen the periods of continual exposure. Of indirect benefit, too, was the installation of factory dentists and the preliminary medical examination which weeded out the more unfit, especially those with dyspeptic or bilious derangements, from entering on a T.N.T. occupation.

Direct measures consisted of steps taken to eliminate contact between the worker and the explosive. These steps included the provision of ample washing accommodation and the supervision of its use, the provision of protective clothing and caps, in some cases provision of factory underclothes to be worn while at work, and the installation of compulsory bathing.

The presence of a resident medical officer in the workshops of the factory served two purposes: (1) to give an eye to the maintenance of cleanly conditions of working, and (2) to pick out those showing signs of T.N.T. absorption and cause their removal from this work before absorption had proceeded to a dangerous degree.

A great elimination of possibilities of contact occurred when steps were taken in the end of 1916 to secure that floors, vessels, and trucks were constantly cleaned, but the multiplication and improvement of mechanical methods of filling was without doubt the biggest factor in the elimination of contact.

The absolute elimination of contact under factory conditions is hardly conceivable, for wherever T.N.T. is handled in bulk there will be a slight and perhaps invisible film of T.N.T. dust on all exposed surfaces. No one who remembers the conditions of working in the summer of 1916 will question that early in 1917 a great change was observed in factory conditions, and by the end of 1917 mechanical working had reached a high state of efficiency. Mechanical arrangements of all sorts had to be thought out and the machines made. This necessarily took time while output could not be delayed.

In any discussion on protective clothing reference must be made to gloves. Their uselessness was referred to by Simeon Snell in 1894 (*B.M.J.*, March 3) and when one realizes the time and care taken to inculcate into medical students an aseptic habit in the donning of gloves it is easy to see why we completely failed to teach workers how to wear gloves in a safe fashion. Experience showed that after a few hours' work the inside of the glove became contaminated with T.N.T. and then the worker's state was potentially worse than had gloves not been worn. Dr. Benjamin Moore reported that he once succeeded in getting the hands chemically clean of one girl by asking her to go to the laboratory before going on and after coming off each working period and by putting on and taking off the protective clothing himself.

Gloves were provided and worn by the million, and in the end their use was universally discarded, although for hot work or to protect the girls' hands from sharp edges of steel their use had to be tolerated.

Clean working necessarily connotes skilled working. The chart (C, p. 24) made by Dr. Castellain at Hereford shows a close parallel between the incidence of T.N.T. sickness and the incidence of accidents.

Between August 3 and August 17, 1918, owing to a great and destructive explosion at Chilwell, the workers at Hereford were

increased by over a thousand. This meant a great leavening by unskilled and untrained workers, whose sickness rate and accident rate showed an immediate and heavy rise.

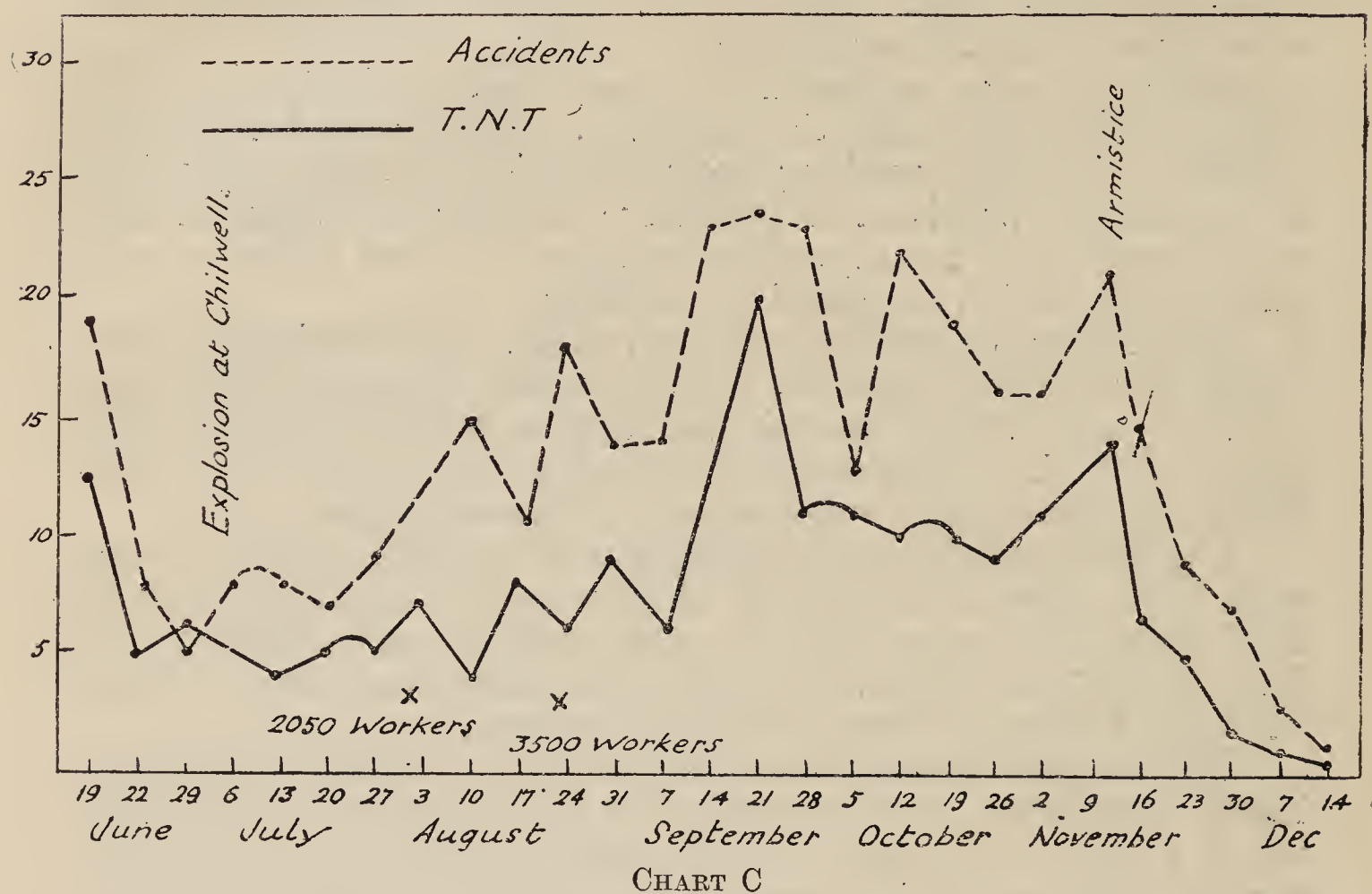


CHART C

VENTILATION.

Ventilation of T.N.T. factories was introduced to effect two objects :

(1) The rapid changing of the air in which the operatives worked, and

(2) To remove dust at its point of origin by a powerful air current.

The oppression felt in any closed room where T.N.T. was manipulated in any bulk was a real phenomenon, and prolonged physical labour was depressing and enervating under such conditions, especially when an artificial high temperature was maintained to facilitate the working of ammonium nitrate.

Every T.N.T. worker is to some extent an absorber of T.N.T. and must in consequence have a microscopic amount of methaemoglobin and NO-haemoglobin in circulation, and an adequate scheme of ventilation is a remedial measure tending to preserve the balance of the haemoglobin katabolism, an undue strain of which Dr. Moore suggested as the underlying cause of the grave ill-effects of T.N.T.

Removal of dust or fume from its place of origin was effected by an exhaust draught produced by mechanical means.

An electric fan operating in a current of air carrying T.N.T. dust particles has explosive and fire risks of its own ; to eliminate this risk an *induced exhaust draught* was generally used. A powerful current of air was blown through a lateral opening at an acute angle into the main duct and this current bore along with it the air in the hinder part of the duct whose opening lay close to the working

process where the dust originated. These induced exhaust draughts were of particular value in the filling of exploder bags; minute quantities of fine dust might fall from the spigot of the bag-filling machine, but with the draught in operation none fell on the worker's hand or on the working bench, and loose empty bags that should not lie exposed to possible contamination in the working area were whisked away along the duct. Processes in which T.N.T. powder was weighed out by hand were safeguarded in a similar manner.

The factory P. was a good example of the effect of ample ventilation in countering minor illness due to T.N.T. absorption; it was situated on the west coast open to the Atlantic breezes. There were four sheds with five or six melting coppers in each. The soiling of floors and benches and the splashing of workers' clothes with molten T.N.T. was common. The spigots of the melting coppers dripped continually, alternation of the employees was unreliable and there were other faults in factory hygiene. Under these bad working conditions jaundice cases were numerous over a short period—17 cases with 7 deaths in nine months July 1916 to March 1917, but the minor sickness throughout was quite a small amount, varying from 2 to 5 cases a week.

ALTERNATION OF EMPLOYMENT.

Alternation of employment has been another safeguard to which great importance has been rightly attached. By removing the worker from contact it lengthens the period during which the factory medical officer can pick out the incipient stages of grave poisoning, it gives time for the elimination of T.N.T. absorbed, and as shown by the table below comparing the experiences of three large and similar national factories, its existence has been a great factor in preventing a large amount of serious disease during the early period while adequate precautionary measures were being installed.

<i>Alternated (1916).</i>				<i>Not alternated (1916).</i>			
Factory	Workers.	Cases.	Deaths.	Factory	Workers.	Cases.	Deaths.
H.	2,011	9	2	C.	3,001	85	25
G.	1,308			L.	2,907		
A.	2,989			M.	1,593		
	6,308	9	2		7,501	85	25

It is obvious that any system of alternation at least doubles the total number of employees in contact with T.N.T., and if 'susceptibility' is a factor there is a risk of more workers developing toxic jaundice unless picked out early by the factory medical officer.

Total Cases of Toxic Jaundice from T.N.T. in Quarterly Periods.

<i>Year.</i>	<i>First.</i>	<i>Second.</i>	<i>Third.</i>	<i>Fourth.</i>	<i>Total.</i>
1916	6 ⁴	16 ⁵	73 ²¹	86 ²²	181 ⁵²
1917	83 ¹²	56 ²⁰	21 ⁸	29 ⁴	189 ⁴⁴
1918	13	6 ²	5 ²	10 ²	34 ¹⁰

Raised figures are *deaths.*)

(5) Theories of Causation of Toxic Jaundice.

In 1916 when T.N.T. work was being heavily pushed and all medical men connected with the problem were being overwhelmed by the flood of work consequent upon the vast numbers of people suddenly absorbed into this new industry, a simple aetiology was everywhere accepted as to the causation of toxic jaundice. Mr. Webster's urine test has shown that practically all workers were absorbers of T.N.T., and toxic jaundice was ascribed solely to the absorption of trinitrotoluene.

With the persistence of toxic jaundice under improved factory conditions the question of an intercurrent factor had forced itself upon the attention of all concerned in the problem, and was first publicly referred to in the meeting of the Section of the Epidemiology and State Medicine of the Royal Society of Medicine of April 12, 1918, and this view is receiving to-day more general acceptance.

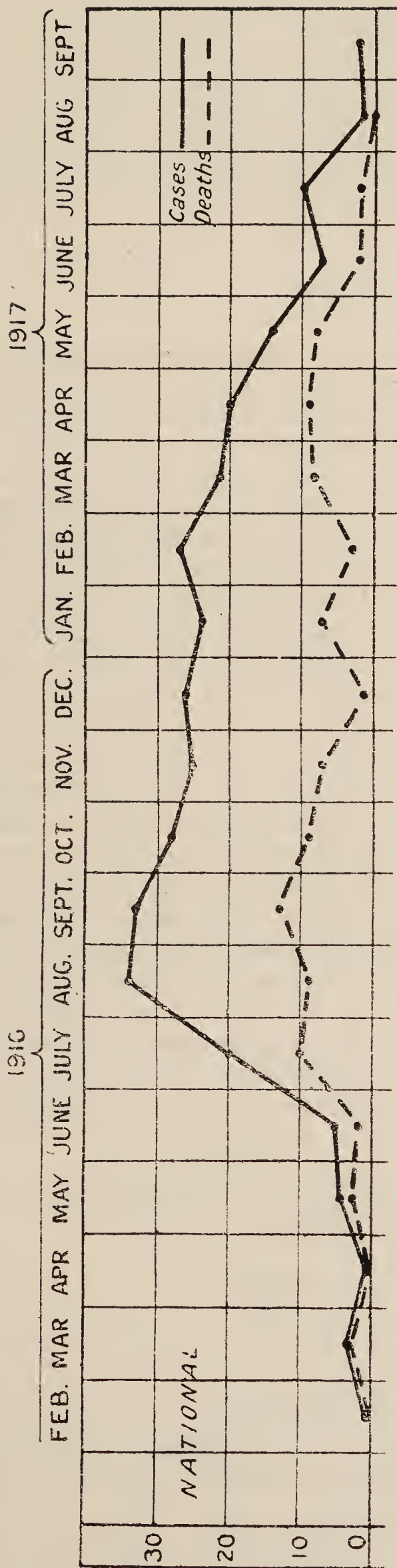
The difficulty of producing portal cirrhosis of the liver in animals by the administration of alcohol has always suggested that an infective factor was always at work in human cases, and the occurrence of cirrhosis of the liver in cows slaughtered for human consumption has always provoked the thought that alcohol was by no means an essential factor. Further, no case has yet been reported of the undoubted production of typical liver changes of jaundice in animals through the administration of T.N.T. Over 100,000 workers have at one time or other been in contact with T.N.T., and yet in all there have only been 404 cases of toxic jaundice reported to the Home Office in the whole country.

A parallel may be drawn between hundreds of thousands of intravenous injections of salvarsan that have been made and the few cases of liver atrophy that have occurred from its administration, from which Dr. Macdonald in the *B.M.J.*, January 19, 1918, favoured the view that in the causation of yellow atrophy we have at least two of the factors at work. The chart (A) printed on p. 19 is very suggestive of an acute infection. The curve below (p. 27) representing the cases of toxic jaundice occurring in the national factories shows an acute onset with a gradual decline such as is seen in curves of epidemic disease, and the Chilwell curve is very similar.

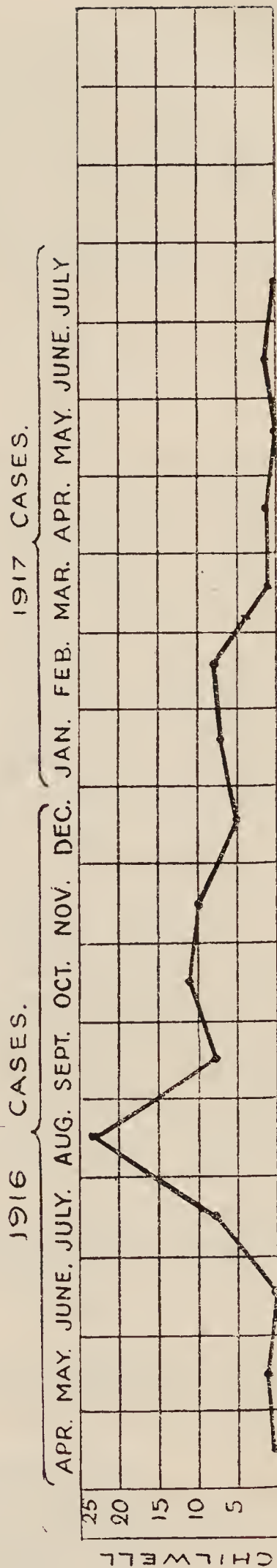
The main outbreak of jaundice cases occurred in Chilwell in August of 1916, in Leeds in September and October of 1916; George-town was first troubled with an outbreak of two cases in December 1916, and one in January 1917; Morecambe had an outbreak of 6-6-3-8 cases in the months of January, February, March, and April respectively, 1917, whereas Hereford had an outbreak of five cases in September, October, and November of 1918. On this one might suggest that the outbreak was comparable with a minor epidemic travelling from place to place. A further factor and one difficult to explain on the basis of a simple aetiology is the LATENT PERIOD observed in so many cases. It is now a common observation that a worker may leave a T.N.T. factory well and yet weeks or months later may be suddenly stricken with toxic jaundice.

It has to be remembered that there were always a certain number

Toxic Jaundice Cases in National Factories.



Toxic Jaundice Cases at Chilwell.



of cases of fatal acute atrophy of the liver occurring in this country, from 43 to 79 yearly, as shown by the Registrar General's returns, 1911 to 1917, and it may well be that in workers whose livers have been sensitized by the action of some metabolic product of T.N.T. bacterial infection that ordinarily would produce a gastric catarrh or a mild catarrhal jaundice would in these cases produce an extensive damage of the liver similar in kind, but much graver in degree, to that found in eclampsia and delayed chloroform poisoning.

APPENDIX

INSTRUCTIONS TO MEDICAL OFFICERS OF FILLING FACTORIES.

1. *Introductory*.—Men and women doctors have been attending at the various filling factories in increasing numbers since it was first realized that all persons coming into contact with T.N.T. in any shape or form could not escape absorbing it through the skin, respiratory or digestive tracts. These doctors should realize the distinction between preventive and curative medicine. They are not stationed there for the purpose of carrying out ordinary medical care of sick people. For coughs, colds, and sprains, for consumption and all other injuries, ailments and diseases there is, or should be, an available panel doctor. The medical officer, on the other hand, is appointed mainly for preventive work.

2. It is of far greater national importance that the incidence of T.N.T. sickness should be lessened, and, if possible, completely stopped, than that this illness when established, together with any other disease that may occur in the factory, should be personally treated. The medical officer should understand the working of the factory from A to Z ; he should be familiar with the housing, habits, rate of wages, and the travelling facilities of the employees ; he should know the relative danger of each part of the filling process, and be able to offer definite advice as to improvement in sanitation, ventilation, heating, or weatherproofing of the various factories and sheds when, in his opinion, they are defective. General suggestions are usually of less value to the management than particular and detailed advice which is the result of skilled observation and consideration.

Broadly speaking, it may be said that the principal duties of the medical officer in T.N.T. factories are five :

- (1) Preliminary medical examination.
- (2) Periodic routine inspection.
- (3) Examination of cases.
- (4) Notification.
- (5) Responsibility for treatment.

All workers in the filling factories, no matter to what department of the Ministry they belong, shall be under the supervision of the medical officers.

3. *Preliminary Medical Examination*.—The first duty of the medical officer is to arrange for the preliminary medical examination

of all workers on their entrance into the T.N.T. side of the factory. The examination should be conducted in a properly lighted, warm, and sufficiently private room, with the applicant stripped to the waist and a shawl or a half blanket around the shoulders. A record must be kept on the authorized card index form, and any subsequent examination should be kept recorded on the back of the same form, or on continuation cards. The importance of this is emphasized by the fact that many conditions such as chlorosis, phthisis, mitral stenosis, aortic incompetence, chronic nephritis, and diseases of far greater rareness are now being met with in the course of this routine preliminary examination. Such cases need not necessarily be rejected as unfit for work, but if employed on T.N.T. the record of their state when first seen would subsequently facilitate a correct diagnosis and might lead to a great saving in compensation. It is advisable to weigh all employees when first seen. Epigastric discomfort and nausea, without or with vomiting, are common symptoms in T.N.T. workers, but are equally common in chlorosis, alcoholic gastritis, and dental dyspepsia. So simple an aid to diagnosis should not be neglected.

4. *Periodic Routine Inspection*.—Many employees, from patriotic or other motives, will work even though they feel far from well, and will even seek to conceal their symptoms. People have been seen at work with conjunctivae chocolate-coloured from deep jaundice. It is only by the frequent inspection of the factory by the medical officer that such cases and others suspected of illness can be suspended or transferred to other work before they either report themselves sick or collapse. Half the value of the medical officer's work is lost if he is content to wait for the employees to report themselves sick. This periodic inspection of all workers, after the initial strangeness has worn off, is welcomed by the employees as evidence of the care taken of them, and it can be rapidly and efficiently done by noticing the colour of the tongue, lips, and conjunctivae and the lack of individual activity and energy at work. Experience shows that all workers very much dislike a fuss being made about their health in the presence of other employees.

5. *Examination of Cases*.—It will be found that among the cases of sickness due to T.N.T. the main types that require absence from work are :

- (1) Dermatitis.
- (2) Gastritis (abdominal pain and vomiting).
- (3) Severe anaemia.
- (4) Jaundice of any degree.

Many doctors have found it advisable to select the more anaemic girls, or those newly employed complaining of gastric pain, and to order them to attend at the surgery two or three times a day for a week or a month to obtain a regular dose of medicine from the nurse. For want of due examination by the medical officer, cases of viscerop-tosis, of chronic salpingitis, of cancer of the stomach, and of hypochondriacism have received compensation and have been seriously treated as cases of T.N.T. poisoning.

6. With careful and frequent medical inspection cases of toxic

jaundice should be rare. If such occur the medical officer should keep in close touch with their progress. Workpeople must never be allowed to leave the factory seriously ill without arrangements being made by the medical officer to keep himself informed as to the progress of the case.

7. The medical officer should attend every post-mortem and inquest whenever possible: (1) to ensure that the *sectio cadaveris* is adequately performed and exclude causes of death other than T.N.T.; and (2) to give evidence as to the precautions taken in the factory to protect the workers.

Owing to the fact that some of the sequelae of T.N.T. poisoning, consequent upon the fatty degeneration and atrophy of the liver cells, may not manifest themselves until some years have passed, it is particularly important that accurate and full records be kept of all sickness in T.N.T. works.

8. *Notifications.*—The Ministry of Munitions require to know the amount of sickness referable to T.N.T., the number of workers suspended or transferred to other work on account of it, and the number of cases of and deaths from toxic jaundice. A weekly return on the special form drawn up for this purpose should be sent to the Medical Adviser at the Ministry giving these details. Any case of or death from toxic jaundice should be immediately reported on its special form to the Ministry. This weekly report is not intended in any way to supersede the statutory obligation devolving on every medical practitioner attending on or called in to visit a patient whom he believes to be suffering from toxic jaundice, contracted in a factory or workshop, to notify it to the Chief Inspector of Factories at the Home Office, London (*Factory and Workshop Act, 1901, s. 73*). These latter reports form the basis of the statistics of toxic jaundice published every month in the 'Labour Gazette'.

9. *Responsibility for Treatment.*—The care of T.N.T. workers may be preventive or curative. With regard to the former a special circular will be issued embodying the principal points which need attention; with regard to the latter, whilst as a rule curative measures will be in the sphere of the panel doctor, the medical officer has a definite share in this responsibility. He should maintain good and intimate relations with the panel doctors who deal with these cases and offer such information, advice, and assistance as may seem advisable. He should also make himself responsible for seeing that the more serious cases receive adequate hospital treatment.

Any suggestions the medical officer can make as to arrangements found helpful at his own factory should be submitted to the Ministry, that they may be submitted for consideration to other factories. Any special dangers that come to his knowledge should be brought to the notice of the Ministry so that in visiting other factories similar defects in organization or care can be looked for and remedied.

10. A medical block is a necessity for the satisfactory performance of the doctor's duties and to economize the time of the workers. There should be separate waiting-rooms for men and women, sufficient privacy for undressing, and sink and water, bench and chemical reagents so that necessary tests can be carried out at once. The examination of new employees and of those with serious symptoms

should be conducted with at least as much thoroughness as obtains in ordinary hospital practice. For proper economy of the medical officer's time, it is advisable that the patient should come before him with his record card filled up as to name, age, weight, and so on ; for this purpose the management may reasonably be asked for clerical assistance. At some factories branch surgeries are provided which can be used for minor dressings and treatment, but the medical officer should work at one central station where he is always available ; experience has shown that this arrangement avoids confusion of records and loss of time for the worker.

The medical care of T.N.T. workers is of the utmost national importance. Men and women come to us ignorant of danger, the latter in the healthiest period of their lives ; we must spare no pains and no professional skill in preventing their leaving us, injured perhaps even for life.

(Signed) W. J. O'DONOVAN, M.D.,
Chief Medical Officer.

MINISTRY OF MUNITIONS,
October 1916.

II. EXPERIMENTS ON THE TOXIC EFFECTS OF TRINITROTOLUENE IN ANIMALS¹

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INTRODUCTION.

IN order to help in the elucidation of some of the problems offered by T.N.T. poisoning, the experiments detailed below were undertaken. They show that, in common with many nitro- and most amino-compounds, T.N.T. has a toxic effect upon haemoglobin, forming a compound no longer capable of carrying oxygen to the tissues. The fact that Webster showed that an amino (strictly a hydroxyl-amino) compound is excreted in the urine of man and rabbits dosed with T.N.T., and that this compound when set free from the glycuronic acid with which it is conjugated is more toxic than T.N.T. itself, suggests that it is the amino group formed in the organism which puts haemoglobin out of action. Such nitro bodies as picric acid (trinitrophenol), which are non-toxic, are, through some chemical or physical peculiarity, probably not reduced in the organism: dinitrophenol is very toxic, and is known to be reduced to aminonitro- or diamino-phenol.

Definite anaemia was produced by large doses of T.N.T., though not of the aplastic type. Benzene being known to cause aplastic anaemia in rabbits, the speculation suggests itself that the toluene nucleus of T.N.T. is responsible for the T.N.T. anaemia.

Liver degeneration, similar in type to, but milder in degree than, the human form, was caused by large doses of T.N.T.

¹ The experiments described in this Report were concluded in August 1917.

Factory observations and experiments having shown that the skin is a highly important channel of absorption in human beings, it is of interest that cats were shown to be capable of absorbing lethal quantities of T.N.T. by the skin.

The experiments were carried out with pure samples of the symmetrical isomer, α -trinitrotoluene, this being the only one used in shell-filling.

For convenience of description the paper is divided into four parts: (1) acute poisoning, (2) chronic poisoning, (3) methods of administration, (4) discussion.

(1) ACUTE POISONING.

Symptoms.

Administration of large doses of T.N.T. to cats led to very pronounced symptoms, whereas rabbits and rats frequently showed very little unless a dose so large as to be quickly fatal were given.

In cats the following are seen: extreme cyanosis, salivation, dyspnoea, diarrhoea, and, if the animal lives a day or two, complete anorexia and rapid wasting.

In rabbits and rats clonic convulsions occur after very large doses, and generally, but not always, indicate approaching death. Slight cyanosis and sometimes diarrhoea occur.

Lethal Doses.

A survey of 13 experiments with *cats* shows that about 0.2 gm. per kilogram body weight is lethal in this species.

Thus, *subcutaneous injection* in oil of 0.21, 0.2, 0.2 gm. per kg. killed cats of 0.38, 2.5, 0.5 kg. wt. respectively, whereas 0.176, 0.17, 0.125 gm. per kg. did *not* kill cats of 0.42, 0.5, 0.62 kg. wt. respectively.

Administration by *mouth in oil* of 0.2, 0.185 gm. per kg. killed cats of 2.5, 0.4 kg. wt. respectively, whereas 0.17, 0.125 gm. per kg. did *not* kill cats of 0.5, 0.58 kg. wt. respectively.

Administration by *mouth of T.N.T. powder* 0.26, 0.25 gm. per kg. killed cats of 0.53, 0.51 kg. wt. respectively, whereas 0.17 gm. per kg. did *not* kill a cat of 0.58 kg. wt.

In *rabbits*, the lethal dose is between 0.5 and 0.7 gm. per kilogram body weight.

A rabbit of 1,500 gm. died three hours after a dose of 0.7 gm. per kg. Convulsions began 20 minutes after the dose. A rabbit of 1,240 gm. survived a dose of 0.5 gm. per kg. It appeared ill the day after the dose, but was well on the third day.

In *rats*, the lethal dose appeared to be somewhat higher than in rabbits.

A rat of 60 gm. died 28 hours after a dose of 0.7 gm. per kg.

A rat of 140 gm. survived a dose of 0.5 gm. per kg. No toxic symptoms occurred. Thus, 0.7 gm. per kg. is probably the lethal dose.

Effects on Liver, Blood, and Bone-marrow.

Liver. I had paraffin sections made from the livers of 14 animals (3 cats, 4 rabbits, 6 rats, and 1 monkey), dying, in periods varying from a few hours to a fortnight, of large doses of T.N.T. Except in those dying very rapidly the doses were repeated, in most cases daily.

Dr. H. E. Turnbull, Pathologist to the London Hospital, was kind enough to examine all the slides for me, and I wish to acknowledge most gratefully the great assistance he afforded me. It had been expected that large doses, single or repeated, would enable one to reproduce in animals the extremely degenerate liver found in fatal human cases of toxic jaundice. This expectation was disappointed, but in the case of four animals, one of each species, fatty degeneration of the hepatic cells at the centres of the lobules was found. The condition was stated by Dr. Turnbull to be similar to that found in early cases of T.N.T. poisoning in human beings, and in one or two rabbits fed with T.N.T. by Dr. Benjamin Moore in the early days of his work on the subject.

A brief account of the experiments on the four animals, giving a partially successful result, is here given :

Cat 32. This was a kitten weighing 320 gm. It had two injections of 0.064 gm. T.N.T. in olive oil (5 per cent. solution), one on each of two successive days. This in the ordinary way would be fatal rapidly. The animal was kept alive in an atmosphere of oxygen, maintained as nearly as possible at a concentration of 70 per cent. (see further, p. 41). The animal was kept in the oxygen chamber about $6\frac{1}{2}$ hours on the first day, and 6 hours on the second. It was very weak and ill when taken out on the second day, and died during the night. Post-mortem, the liver showed to the naked eye a very striking appearance suggesting fatty degeneration. This was confirmed microscopically as stated above.

Rabbit 54. Weight 1,860 gm. Fed on carrots only. Three injections of 0.5 gm. T.N.T. in oil were given during 4 days. The weight rose a little for two days and then fell away rapidly to 1,360 gm. at death. This animal had received 19 days before the first T.N.T. injection, and 25 days before death, an injection of neo-salvarsan. The liver changes were more marked than in any of the other livers examined. There was necrosis at the centres of the lobules and fatty degeneration outside the necrotic areas. The injections of T.N.T. had been given in this case for the purpose of obtaining urine for chemical examination, but the naked eye appearance of the liver was so arresting (pronounced nutmeg type) that a piece was kept for section. Dr. Turnbull gave it as his opinion that the changes were much too acute to be ascribed to the neo-salvarsan 25 days before.

Rat 5. Weight 70 gm. White rat. Given two injections, each of 0.05 gm. T.N.T. in olive oil, with an interval of three days between the injections. Five minutes after the second injection, clonic convulsions occurred. This being nearly always a sign that death would soon occur, the animal was killed with a little chloroform. The fatty changes in the liver were slight but definite.

Monkey. Weight, 4,240 gm. This was the same animal as that referred to under chronic poisoning (see pp. 41-3), but the changes were acute ones, and must therefore be ascribed to the large subcutaneous doses given in the last few days. In the last 14 days nine doses of 0.5 gm. T.N.T. were given hypodermically in oil and a final dose of 0.75 gm.

In the other ten livers examined the only noticeable changes were slight catarrh of the large bile ducts in a cat (12 days' dosing by the skin), and proliferation of the connective tissues round the bile ducts in a rabbit (16 days' injections). In the latter no evidence was found macroscopically or microscopically of coccidiosis, a condition which frequently causes such changes. The remaining eight livers showed no changes of importance.

Besides these 14 livers there were several cases, where for one reason or another no microscopic examination was made, in which a pathological condition would have been almost certainly found. In particular two rabbits showed pronounced nutmeg livers. One of these two had had novarseno-billon 11 days before his first dose of T.N.T. It had five doses of 0.7 gm. T.N.T. in oil subcutaneously, spread over seven days; it died on the ninth day. The other had had no previous treatment. It had five doses on five consecutive days, the first of 0.5 gm., the second of 0.6 gm., the remaining three each of 0.7 gm., subcutaneously in oil. The animal died on the seventh day.

Blood. The effect of T.N.T. on *haemoglobin* varies considerably with the species of animal. Cats show distinct cyanosis with small doses and extreme cyanosis with large doses. Lethal doses kill entirely by oxygen-want. Rabbits and rats require much larger doses than cats before any change in the colour of the blood-pigment is obvious. Large doses cause the appearance of a chocolate-coloured pigment in the blood, and lethal doses probably owe their effect again to asphyxia, the convulsions referred to above being almost certainly asphyxial in origin. The chemistry of the pigment formed is somewhat uncertain, but it is perhaps identical with that formed by the action of nitrites on haemoglobin (Haldane (2)), which may for convenience be called nitrite-haemoglobin. According to Haldane this is identical colorimetrically and spectroscopically with a mixture of one part nitric oxide haemoglobin and three or four parts of methaemoglobin. Methaemoglobin was looked for spectroscopically many times in human beings and in animals. The characteristic band in the red was found only in one case, that of a cat given 0.5 gm. T.N.T. in oil by stomach tube. The animal died in three hours, and the sample of blood giving the methaemoglobin spectrum was taken from the saphena vein shortly before death. The band in the red was faint even in this case, though the blood must have been very heavily loaded with 'nitrite-haemoglobin'.¹

Haldane Test. The test-tube method of laking blood with water and saturating with coal-gas or carbon monoxide was used in several cases. It gave positive results (slight turbidity and a purplish tint

¹ It is difficult to reconcile these spectroscopic observations with any theory that the main change is to methaemoglobin, and additional experimentation upon the altered haemoglobin is evidently desirable.

as compared with the cherry red of the control) in cats and rabbits, in several munition-workers using T.N.T., and in two laboratory workers who were taking daily doses of 0.05 grm. T.N.T. experimentally for a week. The *serum* was examined in Wright's tubes in two cases. One was that of a cat having a series of daily doses of T.N.T. by the skin, the concentration of T.N.T. in the blood being small as judged by the degree of cyanosis; the other was a rabbit having a series of large doses hypodermically. In neither case was haemolysis or bile present. At the post-mortem on the monkey, which was made immediately after the animal had been killed, the blood was noticed to be very distinctly haemolysed. The last dose, two days before death, had been a very large one. In no case was haemoglobinuria observed. This last fact is somewhat remarkable in contrast with some unpublished observations of Dr. Moore's, viz. that rabbits fed with large doses (1 grm. or more) of T.N.T. in powder-form, almost invariably got haemoglobinuria a day or two before death. The difference is presumably due to the different methods of administration, the great majority of my rabbits receiving subcutaneous injections of oily solutions. Evidently the route of administration is a more decisive factor than previous solution of the substance in setting up a high concentration in the blood.

With regard to the *blood-cells*, only a few desultory observations were made until a fortnight before the author was called up for military duties. These early observations were limited to counts of the red cells in three cats and one rabbit; all showed a definite fall in the number of red cells while large doses were being given, and in the case of the rabbit a tendency to rise again on stopping the doses.

A fortnight before the work had to be abandoned an observation was made on a rabbit's blood which led to a hurried series of experiments, the results of which were of considerable interest. The observation referred to was made while examining a blood film for a differential leucocyte count, the latter being undertaken after reading a paper by Selling, showing that benzene in daily doses of 1 c.c. produces in rabbits within a week an enormous fall in leucocytes, and affects the polymorphonuclear cells much more than the mononuclear. That is to say, that the condition of the white cells in benzene poisoning is the same as that found in aplastic anaemia. Quite a number of cases of aplastic anaemia had by this time been reported in munition-workers handling T.N.T., so it was determined to try whether large daily doses of T.N.T. could produce changes in the direction of those found in aplastic anaemia.

Before recording what was found it may be as well to recall the salient features in the blood-picture in a human case of aplastic anaemia; these are (according to Musser (4)): red cell count very low, megalocytes and erythroblasts absent, no poikilocytes, no anisocytosis, haemoglobin low, colour index variable but mostly low, great leucopenia affecting especially the polymorphs.

The findings in the T.N.T. rabbit (No. 34) were as follows: after six doses (first two of 0.4 grm. each, last four of 0.5 grm. each) in ten days the red cells showed very pronounced polychromatophilia, anisocytosis, poikilocytosis, and the presence of nucleated forms; the red-cell count (see Fig. 1) was low, 3,360,000 (normal being 4,500,000).

RABBIT 34.

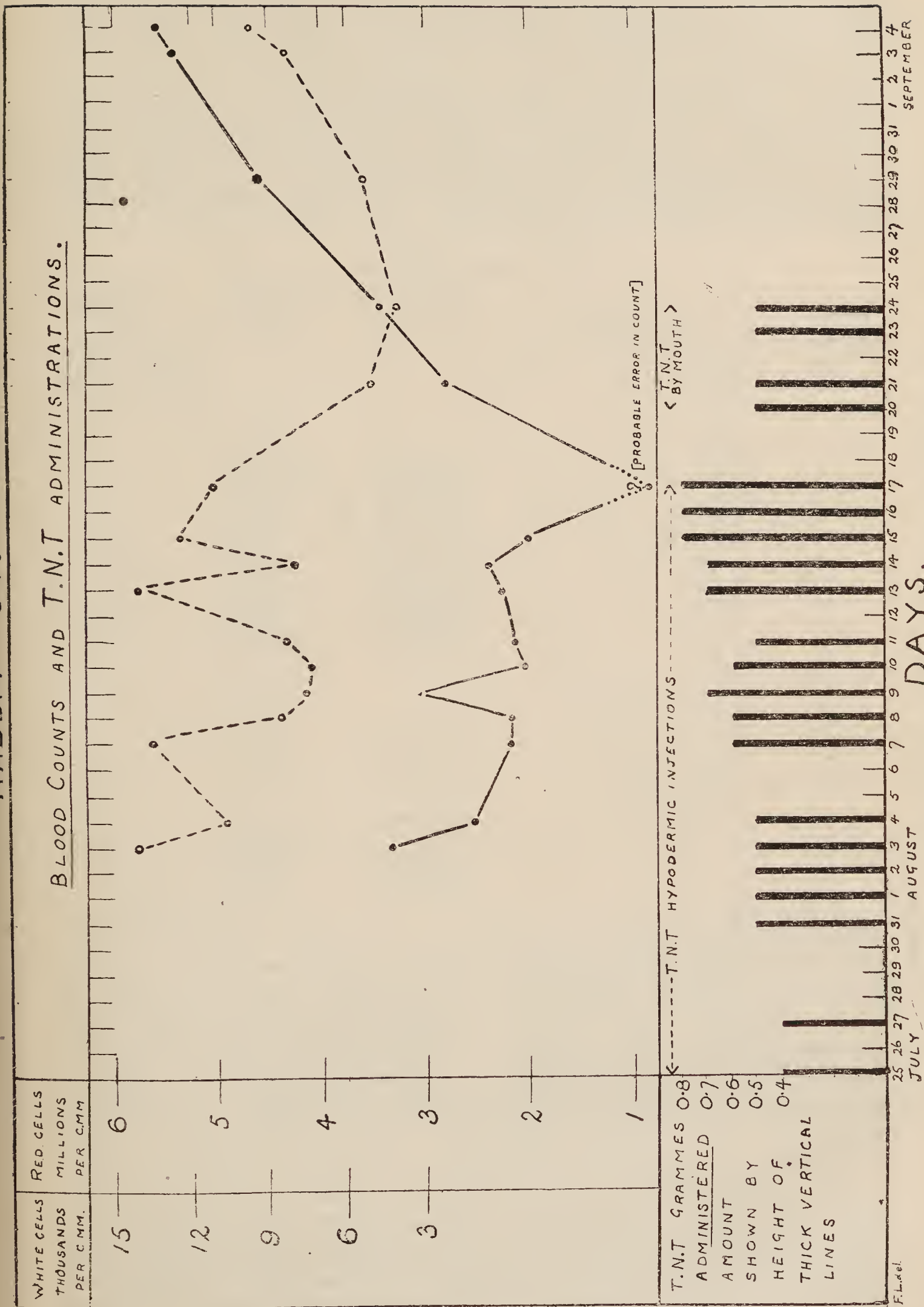


FIG. 1. (Red cells continuous line; white cells broken line.)

to 7,500,000) per cubic mm.; the white-cell count was rather low, but subsequent observations showed it to be fluctuating, the differential count also showed fluctuations. Injections and blood observations were continued for three weeks (Dr. Moore very kindly continued the injections for a time after my departure, and sent me blood films from time to time), and the blood-picture was found to remain as described above, except that the nucleated reds diminished considerably. The animal was then left untreated. Eleven days later the red-cell count was 5,584,000, and the film was practically normal, no nucleated red cells being seen, and only very slight polychromatophilia and anisocytosis. The dosage in this case, as

Blood counts of Rabbit 34.

<i>Date.</i>	<i>Dose of T.N.T.</i>	<i>Red cell count.</i>	<i>White cell count.</i>	<i>Nucleated red cells.</i>
July 25	0.4 gm. subcutaneously	..	..	Number seen while counting 200 leucocytes.
27	0.4 „ „	..	..	
31	0.6 „ „	..	..	
Aug. 1	0.6 „ „	..	..	
2	0.6 „ „	..	..	
3	0.6 „ „	3,400,000	14,000	
4	0.6 „ „	2,600,000	11,000	
7	0.8 „ „	2,100,000	13,000	
8	0.8 „ „	2,200,000	8,000	3
9	0.8 „ „	3,000,000	7,800	7
10	0.6 „ „	2,050,000	7,800	22
11	0.4 „ „	2,200,000	8,100	
13	0.6 „ „	2,300,000	14,200	8
14	0.6 „ „	2,400,000	8,200	14
15	0.8 „ „	2,100,000	12,500	
16	0.8 „ „	..	..	4
17	0.8 „ „	..	11,000	1
20	0.5 „ by mouth	..	..	
21	0.5 „ „	2,700,000	5,000	
23	0.5 „ „	..	..	
24	0.5 „ „	3,500,000	4,000	1
	<i>No more T.N.T.</i>			
29		4,300,000	4,800	
31		..	5,100	
Sept. 3		5,200,000	8,000	
4		5,400,000	9,500	0

usual, was by hypodermic injection of olive-oil solutions of T.N.T., the doses being given daily except on Sundays. The dose was increased gradually until 0.8 gm. was reached; this was given on three successive days. Since a strength greater than 5 per cent. cannot readily be used, and even this requires heating to dissolve the T.N.T., 5 per cent. solutions were always used. This necessitated the employment of fairly large quantities of oil, hence it was thought advisable to do a control experiment with olive oil alone, in order to eliminate the possibility that some or all of the effects were due to the unsaturated fats (see St. Faust and Tallquist (6)) of the olive oil. The control received doses of hot olive oil almost daily for three weeks (17 doses in 21 days), the quantity of oil being the same as that used in the T.N.T. experiment. Blood films were examined on two days before injection, on several occasions during the experiment, and on the last day. The red cells were on all these occasions normal in shape and staining; no nucleated red cells were ever seen. The size of the cells were normal and uniform at first, and

showed only a very slight anisocytosis towards the end. A blood count on the thirteenth day gave 5,332,000 cells per cubic mm., in contrast with 2,240,000 in the T.N.T. rabbit on the twelfth day.

In a third rabbit (No. 39), the blood was examined *ab initio*. On the two days before injection the red-cell counts (see Fig. 2)

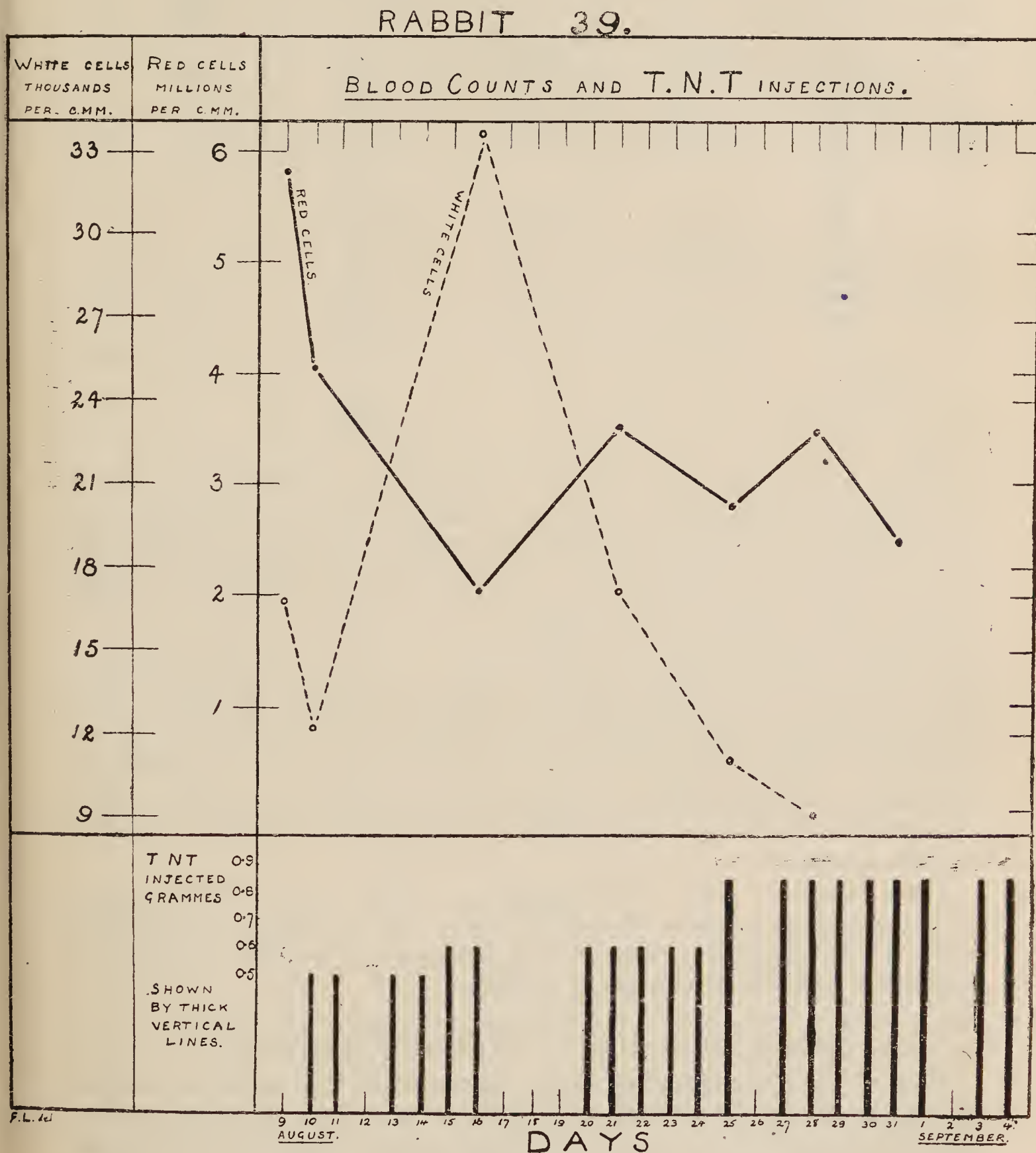


FIG. 2.

were 5,870,000 and 4,120,000 respectively, and the film was normal. After four doses of 0.5 gm. T.N.T. and one of 0.6 gm., slight polychromatophilia, slight anisocytosis, and twelve nucleated reds were seen while counting 200 white cells. The red-cell count was 2,070,000. Dosing was continued for three weeks. The red-cell count remained low and the film similar, except that the nucleated reds diminished in number considerably.

Blood counts of Rabbit 39.

<i>Date.</i>	<i>Red Cells.</i>	<i>White Cells.</i>	<i>T.N.T. Injected.</i>	<i>Weight.</i>
	<i>Millions.</i>	<i>Thousands.</i>	<i>Grammes.</i>	<i>Kilograms.</i>
Aug. 9	5.87	16.85	..	2.16
10	4.12	12.20	0.5	
11	..	..	0.5	2.30
13	..	..	0.5	2.26
14	..	..	0.5	2.28
15	..	..	0.6	2.24
16	2.07	33.60	0.6	
17	..	..	..	2.20
20	..	..	0.6	
21	3.50	17.16	0.6	
22	..	..	0.6	2.30
23	..	..	0.6	
24	..	..	0.6	
25	2.88	11.25	0.85	
27	..	..	0.85	
28	3.43	9.00	0.85	
29	..	..	0.85	
30	..	..	0.85	2.35
31	2.47	..	0.85	
Sept. 1	..	..	0.85	
2	..	..	..	
3	..	..	0.85	
4	..	..	0.85	

These results show that T.N.T. causes in rabbits both red-cell damage and destruction (staining changes and low blood counts), and stimulation of the blood-forming organs (presence of nucleated reds in peripheral circulation). Evidence was also obtained tending to show that regular daily dosing with still larger quantities would produce even more profound effects.

It will be noted at once that the blood condition obtained was far from identical with that found in aplastic anaemia, but it is quite conceivable that blood destruction and bone-marrow stimulation, if continued long enough, would lead ultimately to bone-marrow exhaustion, which seems to be the essence of aplastic anaemia. The diminution of nucleated red cells as the poisoning progressed points in this direction.

Bone-marrow. This was examined in only one acute case, that of the monkey referred to previously. The marrow of the femur was red throughout. Microscopically it showed the presence of numerous cells of all the usual varieties.

Protection from Acute T.N.T. Poisoning by Oxygen.

The symptoms following large doses of T.N.T. in cats pointed so plainly to asphyxia as the chief, if not the only, element causing their rapid death, that it was thought worth while to try whether life could be prolonged by placing the animal in an atmosphere of oxygen, on account not only of its intrinsic interest, but because it was hoped that life might thereby be extended long enough for liver changes to be produced. Kittens were used for this purpose, weighing half a kilogram or less. The method adopted was to inject subcutaneously a large dose (one that would have been fatal in a few hours in the ordinary way) and immediately place the animal in a chamber through which oxygen was passed rapidly from a cylinder until its partial pressure was very high; then this

partial pressure was maintained at the required level by slow bubbling of oxygen. The apparatus used was very similar to that used by Adams (1) in his work on tuberculosis and oxygen.

Of seven kittens used in this manner, three survived the first dose twenty-four hours or more, and were given a second dose, one, two, and three days respectively after the first dose; all these three died within twelve hours of the second dose. Three others died six, twelve, and twenty-four hours respectively after their first dose, in all cases later than would probably have been the case if no oxygen had been used. (One control left under ordinary conditions died in three hours.) The seventh kitten died in $3\frac{1}{2}$ hours, the only one to show no result; this was a weakly kitten to start with, and the weather was very hot at the time.

Thus in six out of seven cases a definite prolongation of life was effected by the use of oxygen, and in three a single lethal dose was recovered from, though a second proved fatal, probably because it was given too soon after the first, though in two or three cases, when the lungs were examined microscopically, commencing oxygen-pneumonia was present. In six of the seven cases an attempt had been made, following Adams, to keep clear of oxygen-pneumonia by maintaining the oxygen percentage at 70 or a little higher.¹ According to Adams, animals can survive for lengthy periods in an atmosphere containing 70 per cent. oxygen or less. The above results do not seem to bear this out, but of course the T.N.T. complicates the question.

With regard to changes in the liver, only two of these seven were examined histologically. One was 'Cat 32' (p. 34), and showed early hepatic degeneration; the other through naked eye a 'nutmeg liver', showed nothing abnormal microscopically.

On the whole the impression was obtained that the experiments would have been worth extending had time permitted.

(2) CHRONIC POISONING.

Symptoms.

Eight prolonged experiments were made with cats and rabbits (three cats and five rabbits), and one with a monkey, extending over three or four months in some cases; small doses being given daily, on alternate days or less often in some cases at certain times when the effects of the poison became too pronounced.

In cats the symptoms were similar to those detailed under acute poisoning—slight cyanosis was nearly always present, slight diarrhoea often occurred. Loss of appetite and weight, if not immediately countered by withholding the dose until recovery, nearly always meant rapid wasting and death.

In rabbits there were commonly no symptoms at all, and the impression was irresistible that rabbits could deal successfully with

¹ The oxygen percentage was determined on two or three occasions each day by means of Hempel's bulbs. It should have been stated above that the injections were done in the morning and, except in the first case, the animal was always taken out of oxygen in the evening after 6 or 7 hours' exposure.

small doses of T.N.T. for an indefinite time, just as the majority of human beings in the shell-filling factories are apparently able to do.

The monkey corresponded in this respect to rabbits and man.

Effects on Liver, Blood, and Bone-marrow.

Liver. The livers of three cats and two rabbits were examined microscopically. The numbers are small, but the general conclusion seems to be that there is a tendency to a mild cholangitis where the doses are not too small.

Cat 15. For four months small doses of T.N.T. were applied to the skin, at first daily, later on alternate days or less frequently. For the first $3\frac{1}{2}$ months the application was made in solution in acetone, the quantity of T.N.T. used varying from 0.1 to 0.5 gm. During the whole of this period the weight, with insignificant fluctuations, kept steady, and the general health was unimpaired. Then application of 0.1 gm. T.N.T. in lanolin was substituted. After four doses of this on consecutive days the animal was so ill that administration was stopped, but despite this, rapid deterioration continued, and death took place a fortnight after the lanolin was commenced, the weight in that short period falling from 2,320 gm. to 1,380 gm. The liver microscopically showed nothing abnormal.

Cat 18. This was a kitten weighing 590 gm. when the experiment was started. Hypodermic injections in oil were given at intervals of one, two, or three days for fifteen weeks. For the first fourteen weeks the dose of T.N.T. varied from 0.01 gm. to 0.025 gm. Then two doses of 0.04 gm. with three days between them caused rapid wasting, and death took place two days after the last dose. The liver microscopically showed nothing abnormal except some scattered deposits of iron-containing pigment.

Cat 12. This was a kitten weighing 580 gm. at the outset. On January 31 a dose of 0.075 gm. T.N.T. dissolved in oil was given by stomach tube. Nothing more was done till February 27 (four weeks) when hypodermic injections of 0.015 gm. T.N.T. in oil were commenced. Six doses were given in eleven days. Anorexia and loss of weight ensued, and the animal was killed six days after the last dose, as death was obviously impending. The liver microscopically showed cellular infiltration of the portal systems.

Rabbit 8a. Nine hypodermic injections of T.N.T. in olive oil were given in eighteen days, the total dosage being 4.5 gm. Five weeks after the last dose the animal suddenly got convulsions and died. The liver was small; microscopically it showed excess of fibroblasts in the portal systems and some fibrosis.

Rabbit 5. Thirteen applications were made on the skin in twenty-five days, the first four being 0.5 gm. T.N.T. in lanolin, the remaining nine the same quantity in acetone. Then a single dose of 1.0 gm. was given, and a week later two small doses with a two days' interval between were given with a view to testing whether the urine reactions corresponded in intensity to those found after similar doses in human beings. They were found to be roughly comparable. Four months after the last dose the animal was fat and well. It was killed and the liver examined. Naked eye a slight coccidiosis was observed. Microscopically cholangitis and some fibrosis of the

portal systems was observed. This may have been due in whole or in part to the coccidiosis.

Another *rabbit* (No. 38) may be included here, though the length of the experiment would place it as intermediate between the acute and the chronic types. In sixteen days nine doses were given hypodermically, varying from 0.2 to 0.5 gm. Diarrhoea and wasting set in and the animal was killed the day after the last dose. The liver showed no coccidiosis, naked eye or microscopically. Proliferation of connective tissue round the bile-ducts was seen.

A *monkey* was given frequent applications on the skin of T.N.T. in acetone over a period of four months. Absorption of a certain amount took place since the urine test for T.N.T. gave a positive result the whole time, but the animal remained quite well. Finally, large doses were given subcutaneously. The liver showed no changes that could be ascribed to chronic poisoning (see p. 35).

Blood. In a rabbit slight cyanosis and a faintly positive 'Haldane test' were present in blood taken four days after a large dose of T.N.T. (0.66 gm.). In man and the cat both cyanosis and a positive Haldane test are present after quite small doses.

The serum of *cat* 15 (see p. 42) was examined in a Wright's tube while under treatment; no haemolysis and no bile were seen. No fall in the red-cell count was found in a cat having small doses of T.N.T. subcutaneously. Slight anisocytosis, one nucleated red cell, and two polychromatophilic cells were seen in a film taken from a thoracic vein at the post-mortem on Cat 18 (see p. 42).

Bone-marrow. At the post-mortem on Cat 18 the femur, humerus, one rib, and a lumbar vertebra were examined. They all showed red marrow throughout.

(3) METHODS OF ADMINISTRATION.

In all but a few of the experiments, administration was either by subcutaneous injection or by application to the skin. The solvent for the injections was in all cases olive oil, the strength being generally 5 per cent. To effect solution in this percentage the oil had to be heated and the injection given while the temperature was still somewhat above body temperature. Where small doses only were to be injected a strength of 2.5 per cent. was used; heat is required here to effect solution, but the T.N.T. does not crystallize out on cooling. Absorption took place rapidly from the oily solution; this was shown not only by symptoms, but by the fact that at the post-mortem on one rabbit some unabsorbed oil failed to give the colour test for T.N.T. with alcoholic potash.

Administration by the skin was used for two reasons (a) because it seemed to be probably the best method of ensuring a continuous entrance into the circulation of small doses of the poison over long periods of time; (b) because experiments in shell-filling factories by Dr. Benjamin Moore (3) and myself had shown the paramount importance of skin-absorption under factory conditions, and it was desirable to establish not only that T.N.T. could be absorbed by the skin (already proved in human experiments) but that fatal doses of T.N.T. could be taken in by the skin. This was shown to

be the case by three experiments with cats. The first of these was a cat (No. 8), weighing 2,400 gm. It received nine doses of 0.5 gm. T.N.T. per dose in lanolin on the skin in thirteen days. Diarrhoea and cyanosis appeared on the third day. Rapid wasting set in and continued till death, when the weight was 1,420 gm.

The second was a cat (No. 14) weighing 2,140 gm. It received four doses only, each of 0.5 gm. T.N.T. in lanolin, on the first, fourth, fifth, and seventh days respectively. It died on the eleventh day. Cyanosis appeared on the fifth day. Wasting set in after the first dose and continued till at death the weight was 1,440 gm. The red-cell count was 5,240,000 per cubic mm. a few hours before death—the normal being 8,000,000 to 10,000,000. The stomach contents were tested post-mortem for T.N.T. and found free, showing none had been swallowed, though it was still present on the skin.

The third cat (No. 15) has already been referred to under Chronic Poisoning.

All precautions were taken in these skin experiments to prevent the animal licking the area of skin used. A coat of stout American cloth, completely covering the trunk and laced up along the back, was fitted, the limbs passing through four holes on the ventral aspect of the garment. The patch of skin used was about 3 inches by 3 inches in size and placed at the middle of one side of the trunk. At first the hair was removed by shaving; later it was found best to be content with clipping it with scissors. It was a matter of some surprise how little resentment the animals, particularly cats, showed to wearing the coat. Lanolin or acetone was used as the solvent in all the skin experiments. The former was far the more effective in promoting absorption of T.N.T., but from its messiness was not well adapted for prolonged experiments. The superior effectiveness of lanolin was almost certainly due to its remaining for an indefinite period in contact with the skin and not to its chemical nature. Acetone evaporated rapidly and left the T.N.T. in powder form, some of it sticking to the skin, but some of it not doing so. For the short period, five minutes or less, during which the acetone remained, absorption was most probably more rapid than in the case of lanolin. A series of experiments was begun, but unfortunately never completed, to test various solvents in relation to their capacity to favour absorption of a dissolved substance by the skin. The leading idea was, after a few preliminary experiments with the ordinary organic solvents, to test the various fats, fatty acids, and alcohols that occur in human skin,¹ using atropine as the dissolved substance and mydriasis as the sign of its having been absorbed. The *modus operandi* was to place one hind foot of a kitten in a wide-mouthed glass vessel containing the solution and hold it in position for a given period facing a well-lit window. The experiments were interrupted when four organic solvents and one other substance had been tested to the extent of giving positive results, and four other substances had so far given only negative results. The results may be summarized thus: the following favour absorption, the first more than the second and so on in descending order—chloroform, ether, xylene, acetone, olive oil; the following all favour absorption a good deal

¹ Or that might come into contact with the skin in munition factories.

less than does olive oil, but cannot be placed in order because only negative readings were obtained with the strengths and times used—alcohol, distilled water, oleic acid, palm oil.

The only points of interest here are that an unsaturated oil (olive oil) favoured absorption more than its corresponding fatty acid, more than a closely related saturated oil, and more than water.

The experiments were suggested in part by an account of some work done by A. D. Waller (9) in 1869 from a different point of view.

The following table summarizes my experiments :

<i>Solvent.</i>	<i>Strength of Solution.</i>	<i>Time of exposure.</i>	<i>Result.</i>
	1 in	minutes.	
Chloroform . . .	1,000	10	Positive.
” . . .	5,000	10	Negative.
Ether . . .	2,000	10	Negative.
” . . .	1,000	10	Positive.
Xylene . . .	250	20	Positive.
Acetone. . .	1,000	10	Negative.
” . . .	500	10	Negative.
” . . .	250	25	Positive.
Olive Oil . . .	500	10	Negative.
” . . .	250	10	Negative.
” . . .	125	25	Positive.
Oleic Acid . . .	100	25	Negative.
” . . .	50	25	Negative.
Alcohol . . .	100	25	Negative.
Water . . .	50	25	Negative.
Palm Oil . . .	50	60	Negative.

It will be observed that the saturated palm oil (mainly consisting of a glyceride of palmitric acid, $C_{16}H_{32}O_2$) in 2 per cent. solution gave a negative result after 60 minutes exposure, whereas the unsaturated olive oil (mainly glyceride of oleic acid $C_{18}H_{34}O_2$) in 0.8 per cent. solution gave a positive result after 25 minutes exposure. This difference is presumably due to difference in solvent power of the two liquids, and therefore difference of surface tension, which latter is of great importance in relation to absorption by living cells.

It was intended to investigate in this manner stearin and stearic acid, cholesterin and lanolin (which consists of esters of cholesterin) ; formic, acetic, proprionic, normal butyric, and caprylic acids, all of which acids may occur in human sweat. If results of any interest had accrued, the solubility of T.N.T. in these solvents and the surface tension of the solutions would have been determined. It was hoped that in this manner some clue would have been found to the problem of the causes of the great differences between the absorption powers of the skins of various human beings.

(4) DISCUSSION.

The fatal forms of T.N.T. poisoning in human beings are (1) ‘toxic jaundice’, a severe hepatic degeneration somewhat like acute yellow atrophy of the liver ; (2) aplastic anaemia.

The majority of the toxic jaundice cases occur after about two months’ exposure to T.N.T. Several chronic experiments with animals lasting three or four months have failed to reproduce this

condition, the dosage being from $2\frac{1}{2}$ to 4 times ¹the maximum dose (per kilogram body weight) that a munition worker can probably take in daily, according to the factory observations of Dr. Moore and myself.

On the other hand, definite liver degeneration (but no jaundice) similar in type to that found in early human cases of toxic jaundice has been caused in animals by large doses of T.N.T., doses much larger than any munition workers are likely to be getting under factory conditions. The very extensive destruction of liver tissue found in human cases has not been attained in these experiments.

It is instructive to compare the cases of T.N.T. with those of other liver poisons, e. g. alcohol, chloroform, tetrachlorethane. The two latter, when they produce recognized liver lesions at all in human beings, produce them rapidly, whereas alcohol produces its effect, if at all, only very slowly. The chloroform and tetrachlorethane conditions are easily reproduced in animals by large doses (see Stiles and Macdonald for CHCl_3 and Willcox for $\text{C}_2\text{H}_2\text{Cl}_4$). It is, on the other hand, correct, I believe, to say that alcoholic cirrhosis of the liver has never been reproduced in animals by prolonged administration of small doses, but that it can be produced by combining the inoculation of certain bacteria with the administration of alcohol (Adami (12), Ramond (5)). Whether the latter observation be confirmed or not, the admitted difficulty in producing 'alcoholic cirrhosis' by administration of alcohol obviously suggests that a second factor is at work in human cases. May not the same be true of the toxic jaundice ascribed to T.N.T.? As possible adjuvant factors the following suggest themselves: bacterial activity, some inherent metabolic defect, a deficiency in diet,² or the presence in excess of some substance in the diet either toxic itself or productive of a toxic substance in the alimentary tract. A case described by Dr. Feldman at the Royal Society of Medicine in February 1917 is strongly suggestive of the 'second factor' hypothesis. The case was that of a woman worker at a T.N.T. factory who stayed away from work eight weeks, and at the end of that time began to suffer from acute gastric symptoms. Two or three days later jaundice manifested itself. If I remember rightly, the patient recovered after a somewhat severe attack of jaundice, too severe to be ascribed to ordinary catarrhal jaundice. The suggestion is that the liver had already been damaged to a considerable extent but was able to cope with all ordinary demands. A severe gastroduodenitis upset the balance and led to manifest signs of the existing liver disease.

With regard to aplastic anaemia, the same hypothesis may be put forward. The observations made on the blood of rabbits on large doses of T.N.T. show distinct blood destruction and marrow stimulation. This, if continued, might lead to marrow destruction,

¹ Thus, e.g., cat 18 received doses on alternate days of 20 to 30 mg. per kg. corresponding roughly to 1 to 1.5 gm. on alternate days for a person weighing 50 kg., or, say, 0.5 to 0.75 gm. per day. Analyses of workshop air and workers' urines indicate quantities of the order of 0.1 to 0.2 gm. daily as the *maximum* amount taken in by all routes under the worst conditions and in cases with the most absorptive skins. This is an outside estimate, a more likely figure being 0.075 gm. for some of the worst cases.

² E.g. carbohydrate-starvation predisposes to 'delayed chloroform poisoning'. See also Rabbit 54, p. 34.

the essential feature of aplastic anaemia. But here again the results were obtained with doses far larger than munition workers could possibly be absorbing, and a second factor hypothesis usefully comes to the rescue.

The relative rarity of toxic jaundice amongst munition workers (somewhere about 1 in 200 T.N.T. workers have, I believe, suffered from the disease) and the still greater rarity of aplastic anaemia is in favour of this explanation of the very partial reproduction of the human disease in experimental animals.

Other possible hypotheses are :

1. That much larger quantities of T.N.T. are being absorbed by some munition workers than are indicated by the colour reactions in their urines. On this hypothesis a large fraction of the T.N.T. absorbed is altered in the body to a much more toxic substance whose end-product in the urine has not yet been detected.

2. That the human cases are due, not to α -T.N.T., but to impurities in commercial T.N.T., such as β - and γ -T.N.T., dinitrotoluenes, nitro-benzenes, nitrous or nitric anhydride, or even some as yet unrecognized gaseous substance formed from molten T.N.T.

With regard to (1), an apparently strong point in its favour is that cats dosed with T.N.T. do not give the urinary colour reaction which man, the monkey, and the rabbit give, while at the same time cats are far more susceptible to poisoning by T.N.T. than the other species.

In this connexion it is of considerable interest that I found that the dinitrotolylhydroxylamine, which Webster showed was the form in which T.N.T. is excreted in human and rabbits' urine, has a lethal dose for cats about one-third of the lethal dose of T.N.T.; for rabbits its lethal dose is about three-quarters that of T.N.T. Webster further showed that dinitrotolylhydroxylamine in human and rabbits' urine is linked up with glycuronic acid, and apparently thereby rendered innocuous. These facts favour the idea that cats form first dinitrotolylhydroxylamine, which, failing to be neutralized, exerts a very powerful toxic action on haemoglobin, and is further changed in this reaction with haemoglobin into an unknown substance, whereas man and the rabbit combine the hydroxylamine, as it is formed, with glycuronic acid, thus neutralizing or at any rate lessening¹ its affinity for haemoglobin.

The question whether T.N.T. is excreted quantitatively as hydroxylamine should permit of experimental test. As a matter of fact, urine for this purpose was more than once collected, and each time through some mistake spoilt or lost. During the course of a large number of human and rabbit experiments undertaken from another point of view, no indication appeared of any failure of correspondence between the dose of T.N.T. taken and the colour reaction in the urine.

As to (2) it may be said, firstly, that no evidence has yet been brought forward incriminating any other substance present as impurity in commercial T.N.T. or formed from it in the process of shell-filling; secondly, the various mixed impurities have been tested by Dr. Moore (3, p. 39), and found no more toxic than T.N.T., and thirdly, that a large proportion of the cases of toxic jaundice in munition workers occurred among those handling the very pure form of T.N.T. used for filling exploder bags.

SUMMARY AND CONCLUSIONS.

1. α -T.N.T. is very toxic to cats, moderately toxic to rabbits, rats, and monkeys.

2. The cat dies after fairly small doses from loss of oxygen-carrying capacity of the blood due to the formation of a derivative of haemoglobin lacking the power of attaching oxygen. The life of a cat injected with a lethal dose can be prolonged by placing the animal in an atmosphere of oxygen; such a dose may even be recovered from altogether.

3. Hepatic degeneration can be produced in all the four species by large doses of T.N.T. In no case has it been produced by chronic poisoning with small doses.

4. Certain blood changes can be produced in rabbits by administration of large doses which probably represent an early stage of aplastic anaemia.¹

5. Fatal poisoning can be produced in cats by administration of T.N.T. by the skin.

6. Comparison is made with poisoning by alcohol, chloroform, and tetrachlorethane, and the general conclusion drawn that an unknown second factor probably co-operates with T.N.T. in the production of the fatal forms of 'T.N.T. poisoning' in human beings.

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¹ The frequent occurrence of cyanosis in munition workers and its presence in rabbits on large doses of T.N.T. show that *some* action on haemoglobin takes place either before or after the formation of the glycuronate, most probably before.

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III. ON THE METABOLISM AND EXCRETION OF 2:4:6-TRINITROTOLUENE

BY T. A. WEBSTER

PREVIOUS to the war only comparatively small amounts of symmetrical trinitrotoluene—for brevity called hereafter T.N.T.—were manufactured on a commercial scale, and as it gave rise to no toxic symptoms among the workers its pharmacology has been very little studied.

Prosser White (1) found that while 90 minims of a 1 per cent. solution of dinitrobenzene, administered hypodermically, was rapidly fatal to a cat, a similar dose of T.N.T. proved quite innocuous. He concluded that T.N.T. was non-toxic.

Jaffé (2) studied the behaviour of ortho-, meta-, and para- mononitrotoluenes in the dog, and found that while the ortho- compound was excreted in the urine conjugated with glycuronic acid, the meta- and para- compounds were found in the urine united with glycocoll and with hippuric acid. The first question studied in this research was how to detect small amounts of T.N.T. or its derivatives that might be found in the urine of workers exposed to the dangers of T.N.T. poisoning. As is well known, many of the nitro derivatives of benzene and its homologues give rise to characteristic colours when treated with a caustic alkali in alcoholic solution, and it was found that while neither the urine itself, nor an extract from it with ether or other non-miscible organic solvent, gave any colour reaction with alcoholic potash, yet if the urine was just strongly acidified with a mineral acid and then extracted with ether, this ether extract gave a pronounced colour with alcoholic potash.

The isolation and identification of this chromogenic body has been previously and briefly described in Professor Moore's (3) monograph on T.N.T. poisoning. A somewhat fuller and amended description is given here.

A priori the most obvious way for the organism to deal with T.N.T. would be either to reduce one or more of the nitro groups to amino groups and excrete the amino body so formed as a salt, or to oxidize the methyl group and eliminate the acid.

The first attempts at isolating the chromogenic body were made with human urine, as it was not quite certain that animals dosed with T.N.T. excreted it in the same form. The urine of a large number of workers from the shell-filling department of Woolwich Arsenal, some 12 gallons in all, was collected. Owing to the large bulk of material ether extraction was not at first resorted to. Attempts were made to absorb the T.N.T. derivative with charcoal; also to precipitate it with lead and basic lead acetates and to extract it by concentration of the urine at low temperatures. All these methods proved unsatisfactory, as the substance is very unstable in the urine, especially if the reaction becomes alkaline.

The following method was finally resorted to :

The urine was extracted once with ether without previous acidification ; this first ether extract was discarded as it contained nothing that gave a colour reaction with the alcoholic potash. The urine was then acidified with half its bulk of 20 per cent. sulphuric acid and extracted twice with ether. The ether extract was well washed with water and dilute sodium carbonate solution to remove acid and finally dried over anhydrous sodium sulphate. It was noticed that, after washing with sodium carbonate solution, the ether extract now gave a beautiful pure blue colour on treating with alcoholic potash, in contradistinction to the varying red to purple colour previously given. This observation was made use of to modify the Webster test and was found to be a great improvement. It was subsequently independently discovered by Tutin (4).

After drying over anhydrous sodium sulphate, the ether was removed by distillation and the somewhat sticky brown residue was investigated. It was found to be partially soluble in hot alcohol ; the alcohol-soluble part gave only a weak reaction with alcoholic potash while the insoluble part gave a most intense reaction. The residue also reduced to some extent alcoholic silver nitrate on warming on the water bath, giving a silver mirror. This suggested the possibility of a hydroxylamino or related body being present, and on looking up the literature it was found that Cohen and Dakin (5) had prepared 2:6-dinitro-4-hydroxylaminotoluene and 2:6-dinitro-4-toluidine by reducing symmetrical T.N.T. with hydrogen sulphide. They investigated the hydroxylamino compound and found that it was decomposed by boiling with strong hydrochloric acid into a white substance melting at 212° C. and characterized by its very sparing solubility in most organic solvents. They concluded from analysis that this substance was 2:6-dinitro-4-aminotoluene and that the other product of reduction, a reddish brown substance melting at 167° C., was 2:6-dinitro-4-toluidine.

These three substances were prepared from T.N.T. by Cohen and Dakin's method and their behaviour with alcoholic potash investigated. It was found that the hydroxylamino compound gave a violet colour, the 2:4-dinitro-6-aminotoluene a pure blue colour ; the 2:6-dinitro-4-aminotoluene gave no colour.

With these interesting results in view the ether soluble mass obtained from the T.N.T. urines was further investigated. On the supposition that some hydroxylamino compound was present, some of the residue was boiled with concentrated hydrochloric acid and two crystalline bodies were identified : (1) a yellowish brown substance melting at 162° C. soluble in HCl and alcohol, and (2) a white substance difficultly soluble in any solvent but finally crystallized from boiling xylol. Substance (1) gave no colour reaction with alcoholic potash ; substance (2) gave the characteristic blue colour.

About this time a paper from the German chemists Anschütz and Zimmerman (6) appeared. Anschütz and Zimmerman showed that Cohen and Dakin were incorrect regarding their investigation of 2:6-dinitro-4-hydroxylamino compound, which on boiling with concentrated HCl gives rise, not to dinitrotoluidine, but to one dinitroaminotoluene (2:6-dinitro) and 2:6-dinitro-4-azoxytoluene.

Substance (2) obtained from T.N.T. human urine as described above was found to be identical with synthetic 2:6-dinitro-4-azoxytoluene. It had the same melting-point (212°C.); mixed with the synthetic substance its melting-point remained the same. It gave the same colour reaction with alcoholic potash; also its molecular weight as determined by Barger's method was the same as that of synthetic 2:6-dinitro-4-azoxytoluene determined by the same method.

It was naturally assumed that substance (1) would prove to be 2:6-dinitro-4-aminotoluene, but while its melting-point was very near that of the synthetic body (162°C. against 168°C.), the mixed melting-point dropped to 120°C. It was not found possible to identify this substance, though it is highly probable that it is a dinitroaminotoluene. The published work on the dinitroaminotoluenes is by no means satisfactory.

Many attempts were made to isolate the hydroxylamino compound from the urine but without success. The reduction of the alcoholic silver nitrate may not improbably have been due to a trace of something else present.

We were also able to obtain the azoxy compound without boiling the ether soluble mass with concentrated HCl.

Rabbits, cats, and a monkey were given T.N.T. dissolved in olive oil subcutaneously and the urines investigated. The injections were kindly given by Dr. H. H. Dale or Dr. G. A. Wyon. From rabbits' urine, as also from the monkey's, we isolated the azoxy compound, but it is not present in cats' urine. The urine of cats that have been given T.N.T. either by the mouth or under the skin give a negative Webster reaction. The urine, however, on acidification and extraction with ether gives a yellow substance soluble in alkalis and evidently an acid; owing to cats succumbing somewhat readily to T.N.T. it was not possible to obtain sufficient urine to proceed with the investigation.

Rats apparently react still differently, as the urine of these animals after feeding on T.N.T. is of a bright pink colour, although it may be quite acid.

It was stated in Professor Moore's monograph that the hydroxylamino compound excreted in the urine (rabbits', human, and monkeys') conjugated with glycuronic acid, and that this broke down on treatment with acid into free 2:6-dinitro-4-hydroxylaminotoluene and glycuronic acid.

Further work on the urine of rabbits that had been given large doses of T.N.T. failed to substantiate this, and, as will be seen from the above, the only substances that have been definitely isolated are (1) 2:6-dinitro-4-azoxytoluene and (2) a yellowish brown substance melting at 162°C. and not identified, although it probably is a dinitroaminotoluene.

On several occasions the urine of T.N.T. rabbits was found to contain more glycuronic acid than that of control rabbits on the same diet. This was not, however, a constant phenomenon. On one occasion I found in a specimen of rabbits' urine a few milligrams of crystalline yellow nodules. These gave the naphtho-resorcin reaction for glycuronic acid, a violet colour with alcoholic potash,

and left a trace of calcium carbonate on ignition on platinum foil. Unfortunately all attempts to obtain more of this substance failed.

Many attempts were made to isolate from the blood and organs of rabbits dosed with T.N.T. the chromogenic substance, but they were always unsuccessful. Even one hour after an injection of 10 c.c. of 10 per cent. T.N.T. in olive oil, when the chromogenic body was beginning to appear in the urine and there must have been some T.N.T. derivatives circulating in the blood stream, a negative result was given to all tests. The chromogenic body—the azoxy compound—must be formed in the kidney.

It is suggested that T.N.T. is first reduced in the body to a non-chromogenic body, the substance found in the urine melting at 162° C. and not identified, and that the greater bulk of this is transformed by the kidneys into the azoxy body, conjugated with glycuronic acid or glycine and excreted.

This work was done in Dr. H. H. Dale's laboratory under the supervision of Dr. Barger, and it is a great pleasure to me to express my indebtedness to both Dr. Dale and Dr. Barger for very much valuable advice and assistance.

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- (2) M. JAFFÉ, *Ztschr. f. physiol. Chem.*, 1878, **47**, 2.
- (3) MOORE, *Medical Research Committee Special Report Series*, No. 11, 1917.
- (4) TUTIN, F., *Lancet*, 1918, ii, 554.
- (5) COHEN and DAKIN, *J. Chem. Soc.*, 1902, **81**, 27.
- (6) ANSCHÜTZ u. ZIMMERMAN, *Ber. d. deutsch. chem. Gesellsch.*, 1915, **48**, 152.

IV. THE FATE OF T.N.T. IN THE ANIMAL BODY

BY H. H. DALE, C.B.E., M.D., F.R.S.

April 29, 1919.

IN the Memorandum on T.N.T. poisoning by Dr. Benjamin Moore, an account is given of experiments carried out by Mr. Webster under Dr. Barger's supervision, to determine the form in which T.N.T. leaves the body. Attention was chiefly directed to the chromogen responsible for the urinary reaction discovered by Webster. The chromogenic substance actually isolated was the azoxy compound formed on partial reduction of one of the nitro groups of T.N.T. The fact, however, that most of the extracts from the urine had undergone treatment which would convert the corresponding hydroxylaminodinitrobenzene into the azoxy compound, led to the suggestion in Dr. Moore's Memorandum that the former was probably the derivative actually excreted. On the other hand, the fact that Webster, as the preceding paper shows, had on occasion obtained the azoxy compound from urine, without treatment such as should have decomposed the hydroxylamino compound, left the matter in some doubt. The point has some significance, in that the hydroxylamino derivative was found by Wyon to be much more poisonous than T.N.T., whereas the azoxy compound is so very insoluble that it can have little significance, at least as an acute poison. It seemed desirable, therefore, to repeat the isolation of the chromogen from urine, under conditions which could not lead to the conversion of the hydroxylamino compound, if present, into the azoxy compound. It will be seen that the result was to identify the chromogen definitely as the azoxy compound. No evidence of the presence of the hydroxylamino compound could be found.

There were other points, of more importance, on which information was not yet available. The chromogen is easily detected in small amounts, and there had been a natural tendency to regard it as the only, or at least the most important, end-product of T.N.T. metabolism in those species in which it appears in the urine. Such an assumption could only be justified strictly by a quantitative determination, showing that a high percentage of the T.N.T. taken into the body reappears in the urine in this form. The fact that in some species (carnivora) no chromogen is found in the urine showed that there were other possible paths for the metabolism of the substance; and the fact that these species were found to be more susceptible to T.N.T. poisoning than those excreting the chromogen suggested that such alternative paths might be more closely associated with the toxic action. The sensitiveness of these species made it very difficult to follow the fate of T.N.T. in them, since they would not tolerate a dosage sufficient to give a reasonable chance of isolating and identifying the end-products in the urine. It seemed more important

however, to attempt to determine, in a species giving the typical Webster reaction in the urine, what proportion of the T.N.T. given was represented by the chromogen excreted, and whether any of the remainder could be identified in the form of other end-products. The rabbit was chosen as the most convenient animal for the purpose, and its tolerance for very large doses of T.N.T. facilitated the investigation. Full-grown rabbits were used, and the T.N.T. was injected hypodermically as a 4 per cent. solution in warm olive oil. The dose given was 0.4 gm. daily to each rabbit. Accidental circumstances caused occasional intermission of the injections for a day or two, but the urine was collected continuously. It will be seen that several rabbits died during the course; others stood the treatment better. No significant macroscopic changes were noted post-mortem in the organs of those which died. The dosage was deliberately pushed to the limit of tolerance, in order to obtain the maximum concentration of end-products in the urine. The urine was taken from the collecting-cages once or twice daily. Samples which could not be worked up immediately were preserved under a layer of ether for a day or two until they could be treated. The chemical investigation was carried out by Mr. F. Tutin, seconded for the purpose to my department by the Ministry of Munitions. The chemical details which follow have been supplied by Mr. Tutin.

Some preliminary investigations were conducted on the urine of rabbits which had received doses of 0.2 gm. of T.N.T. per diem, but since this urine gave only a relatively slight 'Webster reaction' the work was continued with urine from rabbits which had each received 0.4 gm. T.N.T. daily, and this report embodies chiefly the results obtained from the latter.

The following table indicates the treatment of these rabbits and the quantities of urine obtained:

<i>Day.</i>	<i>Number of rabbits.</i>	<i>T.N.T. administered per rabbit.</i>	<i>Volume of urine.</i>	<i>Remarks.</i>
1st	4	0.4 gm.	325 c.c.	
2nd	4	0.4 „	525 c.c.	
3rd	4	0.4 „	870 c.c.	
4th	4	0.4 „	430 c.c.	2 rabbits died.
5th	2	0.4 „	675 c.c.	1 rabbit died.
6th	2	No injection		
7th	2	No injection		
8th	1	0.4 gm.	290 c.c.	2 fresh rabbits.
9th	1	0.4 „		
10th	3	0.4 „	420 c.c.	
11th	3	0.4 „	815 c.c.	
12th	3	0.4 „	935 c.c.	
13th	3	No injection	995 c.c.	
14th	3	0.4 gm.	940 c.c.	
15th	3	0.4 „	970 c.c.	
Amount of T.N.T. injected (deducting for two dead rabbits)			13.2 gm.	
Volume of urine investigated			8,190 c.c.	

All the batches of urine were slightly alkaline to litmus, and were brown in colour when fresh, but they soon became darker and somewhat blackish on keeping, particularly when exposed to light.

The urine, after filtration through paper pulp, was repeatedly extracted with ether until practically nothing further was removed. It was always found necessary, however, to add a considerable

proportion of alcohol to cause the separation of the emulsions formed, so that the extractions were really effected with an ether-alcohol mixture. This first extract may be termed (A).

The urine was then acidified by the addition of one-tenth of its volume of strong hydrochloric acid, and another similar extraction with ether (+ alcohol) carried out. This second extract may be termed (B), and the doubly extracted residual urine (C).

Examination of (A). (Ether extract before acidification.) This ether-alcohol extract did not give any appreciable reaction with 'Webster's test'. It was first washed with successive quantities of water until free from alcohol. The aqueous washings were found to contain some amorphous, brown-coloured material, together with much urea.

The ethereal liquid was then extracted with dilute aqueous sulphuric acid, the acid liquids being subsequently rendered alkaline with sodium carbonate and again extracted with ether. In this way a small amount of an organic base was obtained which was sparingly soluble in water, but readily so in dilute acids. It was, however, entirely amorphous, and the amount was so small that no definite product could be obtained from it.

The ethereal liquid which had been extracted with acid was then washed several times with dilute, aqueous ammonium carbonate, and finally with water. The alkaline extractions removed only some dark-brown tar-like material. The ethereal solution was then dehydrated, and evaporated to dryness. When the residue was dissolved in alcohol and the solution again evaporated to dryness on a water bath, there were left two distinct products which were immiscible; one, a thick, dark brown, tarry product; the other, when warm, a clear yellow oil. These were separated by treatment with light petroleum, in which the oil only dissolved. This oil amounted to about 0.2 gm., and readily became partially solid on cooling. On hydrolysis with alcoholic potash it yielded a soap, and, on acidifying the latter, solid fatty acids were obtained. It would appear, therefore, that this fatty oil was not derived from the olive oil in which the T.N.T. had been dissolved prior to injection, but had been excreted as a result of the action of the T.N.T. on the rabbits. No similar product could be obtained from normal rabbit's urine.

The dark brown tarry product from which the oil had been removed by means of petroleum was treated with dry ether, when it was found that a portion of it had become insoluble, and this was removed as a dark brown, amorphous powder. The material, soluble in ether, which was small in amount, was dissolved in a little benzene, and the solution allowed to evaporate slowly. A very small quantity of crystals then slowly formed which were drained on a tile from the thick, dark brown mother liquor. The small amount of impure crystals thus obtained gave a strong 'Webster reaction'. They appeared to consist of a trace of the chromogen which had existed in the urine in the free state. It will be seen later that the chromogen is the azoxy compound.

Examination of (B). (Ethereal extract of urine after strong acidification.) The combined ethereal extracts of the acidified urine

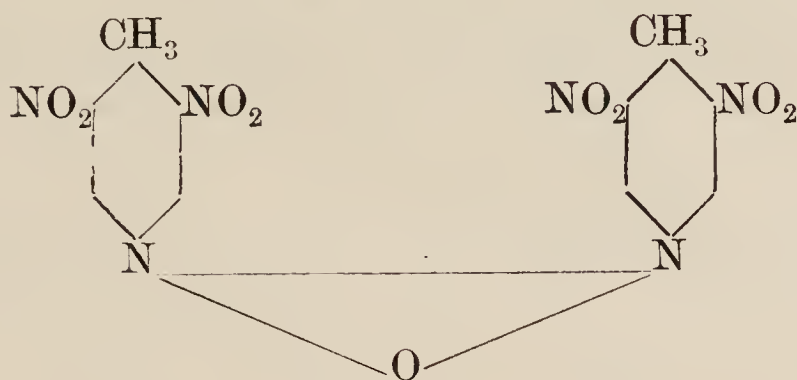
gave a strong 'Webster reaction'. They were washed with water, but this removed only alcohol and a little amorphous colouring material. The washed ethereal liquid was then extracted with aqueous ammonium carbonate when about two-thirds of the material dissolved in it was removed. The alkaline extracts, which were brown in colour, were acidified, and extracted with ether. On removing the solvent a dark-coloured residue was obtained which appeared to consist of organic acids. It was esterified by means of methyl alcohol and dry hydrogen chloride, and the resulting esters isolated. On steam distillation of the latter the greater portion passed over as a colourless oil, which was identified as methyl benzoate. The non-volatile portion consisted only of a small amount of very dark brown tar, from which nothing definite could be isolated. A sample of normal rabbits' urine, when subjected to a treatment identical with that applied to the urine from the T.N.T. rabbits, yielded no benzoic acid. It would appear probable, therefore, that the administration of T.N.T. had prevented the formation of hippuric acid and allowed the benzoic acid to be excreted in the free state. The effect is suggestive of an injurious action on the kidneys, inhibiting the normal synthesis of hippuric acid in those organs.

The ethereal solution, which had been extracted with aqueous ammonium carbonate, was washed, dried, and concentrated to about 200 c.c. On keeping this liquid it slowly deposited a dark brown, amorphous powder, which was removed by filtration, when it was found to be insoluble in all the usual solvents. It dissolved readily, however, in concentrated sulphuric acid, and appeared very similar to the insoluble, dark-coloured products which are formed from T.N.T. by the action of light or of certain bases.

The ethereal filtrate was evaporated to dryness, and then treated with dry ether, when a further amount of the above-mentioned, insoluble, amorphous product separated, and was removed by filtration. These operations of evaporation, re-treatment with dry ether, and filtration, had to be repeated a considerable number of times before the separation of amorphous, insoluble material ceased. This amorphous powder represented eventually about half the original neutral material which had been in the ether extract. It would appear to be derived from the T.N.T., since it deflagrated on heating, but nothing crystalline could be obtained from it.

The final ethereal filtrate from the amorphous powder was treated with animal charcoal, it having been ascertained in previous experiments that the crystalline substances which it contained were not removed from the ether by this treatment. The filtrate was then evaporated completely and the residue dissolved in about 50 c.c. of hot alcohol. On thoroughly cooling this solution a quantity of brown, semi-crystalline solid separated, which appeared almost insoluble in cold alcohol. By further treatment with animal charcoal in benzene solution, and several crystallizations alternately from small quantities of the latter solvent and large volumes of hot alcohol, it was obtained in small, colourless leaflets, and was identified as the azoxy compound, being identical with the compound formed by heating 4-hydroxylamino-2 : 6-dinitrotoluene with concentrated

hydrochloric acid. The total amount isolated was 0.40 gm.—3 per cent. of the total amount of T.N.T. administered. It will be noted that this compound was extracted without subjecting the extract to any treatment which could lead to its formation from a pre-existing hydroxylamino compound.



The original alcoholic filtrate from the crude azoxy-compound was evaporated until it formed a thin, very dark brown syrup. On keeping the latter, crystals slowly commenced to form, but their separation was not completed until after several weeks' standing. The thick, dark-coloured syrup containing the crystals was then placed on a porous tile and allowed to drain in an atmosphere saturated with alcohol vapour, when a yellow, crystalline solid was left on the tile in a fairly clean condition. After re-crystallization from a small volume of absolute alcohol this substance melted at 154–155°, but after several crystallizations, alternately from small volumes of absolute alcohol and hot benzene, the melting-point was raised to 171°. The total amount obtained from the quantity of urine under treatment was 0.52 gm.—4 per cent. of the total dosage of T.N.T.

A very small quantity of another substance, of a much lighter colour, was contained in the mother liquors, but this could not be isolated. The total amount of crystalline derivatives of T.N.T. isolated in a state of purity therefore amounts to almost exactly 7 per cent. of the weight of the T.N.T. administered to the rabbits.

The identification of this yellow compound, representing a larger proportion of the T.N.T. administered than the Webster chromogen, was obviously a matter of some interest. It is probably identical with the substance obtained by Webster, which he also found to be very similar to but not identical with the only known amino-dinitrotoluene (see p. 51). Unfortunately its examination has been attended with difficulties which have prevented its identification with complete certainty. Its properties suggested that it might represent a further stage of reduction than the azoxy-compound, one nitro-group of the T.N.T. being fully reduced to an amino-group, thus forming an amino-dinitrotoluene. Two such compounds are possible as products from the symmetrical T.N.T.—2-amino-4:6-dinitrotoluene, and 4-amino-2:6-dinitrotoluene. These both occur in the literature of the subject, having been described by Holleman and Böseken. According to these authors the 2-amino-compound melts at 155°, and this was the melting-point of the compound from the rabbits' urine when first obtained. On further purification, however, the melting-point of the product

from urine rose to 171° , which is that of 4-amino compound, as determined by Holleman and Böseken. A sample of the latter was prepared, and was found to be very similar in its solubility and general properties to the product from urine, but the melting-point of the two, when mixed, fell much below the temperature of 171° , at which both individually melted when pure.

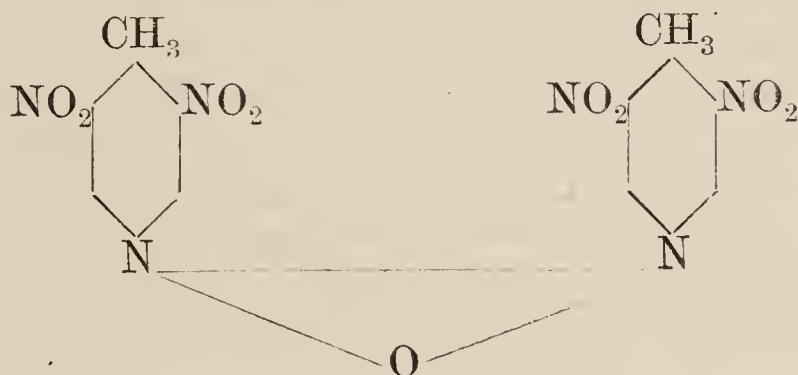
So far the evidence would seem to exclude either formula as that of the product from urine, but a further investigation of the second material obtained by Holleman and Böseken's method, and described by them as the 2-amino-dinitrotoluene, revealed the fact that it was really an impure sample of the 4-amino compound, the melting-point rising gradually with repeated purifications until it reached 171° . All attempts to prepare the genuine 2-amino compound by synthesis failed, so that the possibility remained open that the product from urine had this structure. So far only the 4-amino structure was definitely excluded.

An accurate analysis would have greatly strengthened the attribution of such a constitution to this body, but unfortunately its explosive properties foiled repeated attempts to obtain trustworthy figures by combustion. Determinations of the molecular weight, on the other hand, made by Barger's microscopic method, and by the cryoscopic method in glacial acetic acid, were quite consistent with such a formula. The values obtained by the two methods were 200 and 209 respectively, that theoretically required for an amino-dinitrotoluene being 197.

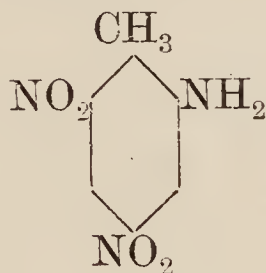
As another possible method of identification, the removal of the amino group, if present, suggested itself; the dinitrotoluene resulting should have been identifiable without difficulty. The conditions were first determined under which this transformation could best be effected with the synthetic 4-amino compound, and a good yield of 2:6-dinitrotoluene was obtained from this. The 5-amino-2:4-dinitrotoluene (prepared from γ -T.N.T.) was treated by the same method and with difficulty yielded a moderate amount of 2:4 dinitrotoluene, with a small amount of a substance having the properties of a dinitrophenol. The 3-amino-2:4-dinitrotoluene (obtained from β -T.N.T.) was quite unaffected by the same treatment. It was evident, therefore, that the method, consisting of diazotizing and pouring the reaction product into boiling absolute alcohol, though perfectly successful with the 4-amino compound, was varyingly successful with those having the amino group in other positions. On treating the product from urine, the diazotization appeared to proceed normally, but on working up the product obtained by pouring into alcohol, the main portion was found to consist of a substance having the properties of a dinitrophenol. It formed hard yellow crystals, which darkened on exposure to light, and dissolved in aqueous sodium carbonate to a deep yellow liquid. A small amount of neutral material was also formed, but contained at most a trace of a dinitrotoluene. The greater part of this small neutral residue consisted of a nearly colourless crystalline compound (m.p. 204°), which gave a deep, but transient red colour with alcoholic potash.

Since neither 2-aminodinitrotoluene itself, nor the corresponding

phenol, with the hydroxy group replacing the amino group, was known or could be prepared, the identification of the yellow substance from the urine remained incomplete. At the same time there was established a strong probability in favour of its being the 2-amino compound. If it is so, it would appear that T.N.T. in the rabbit can undergo reduction in one of two positions. If the NO_2 group in the *para* position relative to the methyl is reduced, the reduction proceeds only as far as the formation of the azoxy compound



This is excreted in the urine as a water-soluble complex, probably, as suggested by Webster, with glycuronic acid. It is only when this is decomposed by the hydrolytic action caused by acidifying the urine, that the free azoxy compound can be extracted with ether and give the characteristic colour reaction of the Webster test. If, on the other hand, reduction takes place on a nitro group in the *ortho*-position relative to the methyl group, the reduction is apparently complete, producing 2-amino-4:6-dinitrotoluene



which gives no colour-reaction with alcoholic potash, but is excreted, like the azoxy compound, as a complex which must be hydrolysed before extraction with ether becomes effective.

A somewhat larger proportion of the T.N.T. leaves the body as the (?) aminodinitrotoluene than as the azoxy compound; but the quantities obtained pure of the two together only amounted to 7 per cent. of the T.N.T. administered. The fact that these compounds are not very stable makes it certain that the true yield was somewhat greater; but even if it be assumed that the whole of the amorphous material, removed from the ethereal extract (B) in the process of purification, represented products of decomposition of these two bodies, this would do little more than double the combined yield. There would still be some 85 per cent. of the T.N.T. administered to be accounted for in other ways. Without doubt a large proportion of it was excreted in the form of highly coloured and unstable compounds, which on any attempt at isolation formed tarry and resinous substances from which nothing could be isolated in a state of chemical purity. These dark tars certainly represent the greater part of the T.N.T. absorbed, and their presence suggested

that most of the T.N.T. is changed in the body into products less stable and more prone to oxidative decomposition than either of the two derivatives isolated—the azoxy compound or the (probable) aminodinitrotoluene. The presence of such more readily oxidized derivatives was suggested also by the red staining of animal's fur by the urine—a reaction given by neither of the identified derivatives—and by the darkening of the urine on keeping. The derivatives with such properties most likely to be formed in the body from T.N.T. are those in which two nitro groups are reduced—diamino-mononitrotoluenes. Such compounds are known to be extremely unstable; attempts by chemists to prepare them synthetically have been frustrated by the formation of tarry decomposition products. If they occurred in the urine, no ordinary method of isolation would be likely to succeed. It seemed possible, however, that they might be obtained as derivatives, if the sensitive amino groups were protected, as by acetylation. If such compounds were present, the basic properties due to the presence of two amino groups would probably prevent their extraction from the acidulated urine by ether, and they should remain in the residual urine (C).

Examination of (C). Successive quantities of this residual urine were evaporated to dryness *in vacuo* and treated with acetic-anhydride in the presence of pyridine. The resulting product was extracted with ether, but in each case only a trace of crystalline substance could be obtained, which was much too small to permit of any attempt at identification.

The conversion of T.N.T. in the body into more highly reduced compounds remains, therefore, nothing more than a suggestive possibility. If such compounds were formed their presence might have much significance for the toxic action. With such further reduction the product would approach more nearly the structure of toluylenediamine, the toxic action of which on the blood and the liver is one of the classical observations of pharmacology. The only definitely isolated end-products, however, are still the azoxy compound, which is the Webster chromogen, and another crystalline substance, which is probably, but not certainly, 2-amino-4:6-dinitrotoluene.

Some considerations as to the significance of the Webster test are suggested by these observations. It has been recommended and used as a signal indicating danger of T.N.T. poisoning. It is a definite indication that T.N.T. in recognizable quantity is being absorbed, and so far its use as an index of danger is justified. If the reaction is prevalent and strongly marked in the urines of workers in a factory, it may serve as an indication for increased precaution against absorption of T.N.T. On the other hand, its appearance in the urine of a member of a group known to be exposed to T.N.T. absorption cannot be properly regarded as an index of individual danger of poisoning. The azoxy compound responsible for the colour-reaction is very insoluble in all kinds of solvents—so insoluble that no satisfactory method of testing its toxicity has been devised. This property alone makes it probable that it is less poisonous than T.N.T. itself. Its appearance in the urine is an index that it is being properly dealt with by the body, by condensation with

glycuronic acid and excretion. It may be suggested that the *absence* of this reaction from the urine of a patient who is known to be absorbing T.N.T. would be the really ominous sign. The evidence is so incomplete that no definite statement is justified; but the weight of probability seems certainly to be in favour of the view that the danger of serious intoxication will increase with the tendency to the formation in the body of more highly reduced derivatives of T.N.T., and that excretion as the azoxy compound represents a stoppage of reduction at the safest point. In appraising the significance of the Webster reaction in an individual case, its bad significance as an index of T.N.T. absorption must be balanced against its probably good significance as an indication that the body is dealing with what is absorbed in the safest way. It must be added that no data are available to show how far the conclusions, based on experiments with massively dosed rabbits, as to the small proportion of the T.N.T. excreted in this form, can be applied to men absorbing relatively small amounts.

V. SUMMARY OF AN EXPERIMENTAL INVESTIGATION INTO T.N.T. POISONING

BY P. N. PANTON, M.B., B.C., Cantab., *Clinical Pathologist to the London Hospital*, and HAROLD E. BATES, M.D., Baltimore, U.S.A.

IN the course of the investigation of cases of poisoning among T.N.T. workers certain problems called for experimental work on animals.

First as to the exact nature of the toxic substance. T.N.T. itself was originally considered to be non-toxic, and attention was directed to impurities and decomposition products of T.N.T.

One of the first possible sources of poisoning to which attention was called was the inhalation of fumes given off by T.N.T. Nitrous fumes in sufficient amount to be detected by the sense of smell are given off by T.N.T. Dr. Benjamin Moore (*Proc. Roy. Soc. Med.*, Jan. 1917) pointed out that workers in processes which involve the inhalation of much larger quantities of nitrous fumes than in the handling of T.N.T. are not affected by either jaundice or anaemia. In this connexion one of us with Dr. Vaidya exposed rabbits and white rats to nitrous fumes in a fume chamber over long periods of time. The percentage of NO in the chamber was far greater than ever could occur in any T.N.T. process. It was found that the animals even with small quantities of gas showed cyanosis, and gave a positive Haldane's test for methaemoglobinaemia. Repeated blood examinations were made and in no case was there any anaemia. The animals showed no jaundice and blood serum taken at intervals showed no bile. At post-mortem examination no changes were found in the livers comparable to those in cases of T.N.T. poisoning.

The following experiments were performed.

A rabbit weighing 4 lb. 4 oz. was placed in a chamber of concentrated NO fumes for two hours at a time. It had a total of thirty hours in the chamber, the experiment lasting over a period of three months. Five blood examinations were made, there was no appreciable change in the red-cell count, haemoglobin percentage, white-cell count, or differential. No bile was present in the blood serum. The animal was cyanosed after each exposure to the fumes, and Haldane's test for methaemoglobinaemia was positive. At post-mortem examination the liver, kidneys, spleen, and bone-marrow were normal. The lungs were deeply congested and microscopical examination of sections showed areas where the alveoli were filled with blood, epithelial cells, and a fair number of polymorphonuclear cells.

A second rabbit, weighing 5 lb. 4 oz. was placed in the NO fume chamber for two-hour periods nearly every day for nine weeks, the experiment extending over a period of three months. Five blood examinations were made and no appreciable change in the counts was found. No jaundice was seen, and no bile was found in the blood serum. Cyanosis was present after each exposure and Haldane's test was strongly positive. Post-mortem examination showed the liver, kidney, spleen, and bone-marrow normal. The lungs showed areas of congestion, and the microscopical picture was similar to that of the first rabbit.

Two rats were also treated in a similar manner and showed no blood changes and the viscera were normal at post-mortem examination.

The only substance in high explosives other than T.N.T. which is used in large quantities is ammonium nitrate, which is mixed with T.N.T. up to a proportion as high as 80 per cent. Ammonium nitrate, however, is believed to be quite harmless.

A rabbit weighing 4 lb. 7 oz. was given by hypodermic injection 5 gm. ammonium nitrate dissolved in distilled water at each injection. Fifteen doses in all were given or a total of 30 gm. The experiment lasted two and a half months. Three blood examinations were made. There was no change in the red-cell count, white-cell count, or differential. There was no jaundice and no bile in the blood serum. At post-mortem examination the organs were normal throughout.

Leaving out of account certain substances, such as tetranitromethane, which occurs in very small quantities in commercial T.N.T. and the toxic action of which has been investigated by others, the obvious conclusion is that T.N.T. itself is the toxic substance. The direct proof of this is, however, difficult to obtain. The main difficulty has been the original failure to reproduce the major lesions of T.N.T. poisoning in animals, and the more recent experiments have been only partially successful. In the first series of these experiments carried out by one of us and Dr. Vaidya commercial T.N.T. was used. Five rabbits were injected with varying amounts of T.N.T. dissolved in toluol. Repeated blood examinations were made and post-mortem notes were taken. In none of these experiments were the major lesions of T.N.T. poisoning reproduced.

We will give the details of these very briefly.

A rabbit weighing 4 lb. was given by hypodermic injection 50 mg. of T.N.T. daily with certain intervals. A total of 1.15 gm. of T.N.T. was given over a period of two months. Six blood examinations were made. There was no marked change in the blood picture, no jaundice and no bile in the blood serum. Because of a subcutaneous abscess the animal was killed. At the post-mortem the organs were normal throughout.

A rabbit weighing 3 lb. was given a total of 1.7 gm. of T.N.T. in doses ranging from 50 mg. up to 500 mg., over a period of two months. Six blood examinations were made with no appreciable change in the counts. No jaundice was present and no bile in the serum. Organs were normal throughout at post-mortem.

A rabbit of $3\frac{3}{4}$ lb. was given a total of 0.9 gm. of T.N.T. in 100 mg. doses over a period of three weeks. The animal developed an abscess at the site of injection and was killed. Four blood examinations were made with no change in the counts. There was no jaundice nor bile in the blood serum. The organs were normal on post-mortem examination.

A rabbit of 6 lb. was given one dose of 500 mg. T.N.T. and died ten days later. The organs were normal at post-mortem examination. One blood count only was done, which showed nothing abnormal.

In attempting to explain the fact that such a small percentage of T.N.T. workers suffer from the poisonous effects, it was suggested that the poisoning might arise by a process of sensitization, by which a worker receiving small doses of the poison at intervals might render himself sensitive to a subsequent dose. In this connexion it was attempted to sensitize a rabbit by alternating small and large doses. This, however, failed to have any other effect than the regular mode of administration.

A rabbit weighing 3 lb. 10 oz. was given a total of 2.4 gm. of T.N.T. This was given in alternating doses of 50 mg. and 500 mg. (with one dose of

750 mg.) at intervals of ten days. Three blood examinations were made, with no change in the blood picture. There was no jaundice and no bile in the blood serum. The organs were normal throughout at post-mortem.

The animals used in the earlier experiments were rabbits, guinea-pigs, and rats. That the metabolism of the drug varies in different species was proved by Mr. Webster and Dr. Wyon working under the auspices of Dr. Dale and Dr. Barger (*loc. cit.*). It was subsequently shown that cats were more sensitive to T.N.T. than rabbits. All of our later experiments have been made upon cats, and while the results have not been particularly encouraging, it has been possible to reproduce some of the liver changes, with typical central necrosis of the liver lobules though never the massive necrosis found in the human cases, and one typical case of aplastic anaemia was developed.

A possible explanation of the small proportion of workers affected, and the difficulty of reproducing the lesions in animals was that of variation in the composition of the drug itself. Attention was therefore directed to the different toxic effects of the three T.N.T. isomers. The great bulk of the powder consists of the symmetrical or α -T.N.T. The β - and γ -T.N.T.s vary in amount in different samples from a mere trace to 4 per cent. in an exceptional sample analysed by Mr. F. Tutin. We therefore made several experiments to determine the effect of the three T.N.T. isomers with the advice and assistance of Dr. Dale.

Another suggestion, made by Dr. Laidlaw, was that the introduction of colon bacilli in addition to the T.N.T. might succeed in reproducing the liver changes. This also we tried.

We wish to call attention to the fact that the number of experiments is wholly inadequate for conclusive results, but the amount of work required for even a small number of experiments was more than could be accomplished in the time at our disposal.

In all thirteen cats were used. As the T.N.T. was given dissolved in toluol, one cat was given toluol alone as a control.

It was particularly attempted to reproduce aplastic anaemia or the liver changes or both. The method of administering the drug was the same in all cases. The different T.N.T. isomers were dissolved in toluol and given by hypodermic injection beneath the skin of the lumbar region.

The general effect of the drug may be considered first. Where the drug was given in small repeated doses over a considerable period, loss of weight was a constant and well-marked feature. The animals became drowsy, refused their food, lost weight rapidly and died. Frequent intermissions were therefore necessary. Especially was this the case in cats treated with α -T.N.T.; although the lethal dose for the cat of T.N.T. β or γ is smaller than that of α , repeated small doses of β and γ seem to be better tolerated than in the case of α .

Cyanosis was noticeable in the early stages of chronic poisoning, but this was later obscured by the yellow colour which developed.

Jaundice, as evidenced by a yellow colour of the skin and mucous membrane, was constantly present, in varying degree. The skin and subcutaneous tissue at post-mortem were in some cases deeply yellow. This yellow colour, however, as pointed out by Dr. Dale,

was not due to the presence of bile pigments in the blood, but to a combination of the T.N.T. with the body proteins producing a yellow reaction. The blood serum was deeply yellow in all animals treated with β - and γ -T.N.T. and was not noted in any of the cats which were given pure α -T.N.T.

Local necroses at the site of injection were common. These necroses were usually sterile, but occasionally an extensive abscess developed and the animal had to be killed.

In estimating the effects of these drugs upon the histology of the organs we availed ourselves of the kind assistance of Dr. H. M. Turnbull. In two instances Dr. Turnbull made the complete post-mortem examinations and in other cases examined the sections for us. Dr. Turnbull's reports and remarks are placed in inverted commas and initialled.

The effect of the drug on the *condition of the blood*. In eight cats periodic blood examinations were made, and in seven no specific blood changes were produced. The eighth case is of considerable interest.

A cat was given small doses of γ -T.N.T. by Dr. Dale over a period of from nine to ten weeks. 50 mg. given daily with remissions. The first blood examination showed:

Red cells	6,750,000 per c.mm.
White cells	22,800 per c.mm.

Differential count:

Polynuclear neutrophils	89.5 %
Small lymphocytes	5.0
Large lymphocytes	0.5
Large hyaline	5.0

One normoblast was seen among 200 leucocytes. There was some polychromatophilia. This is a comparatively normal blood count for a cat.

Six days later the cat was very ill, and the blood examination at that time showed:

Haemoglobin	18 %
Red cells	1,937,500 per c.mm.
White cells	600 per c.mm.

Differential count:

Polynuclear neutrophils	8 %
Small lymphocytes	58
Large lymphocytes	18
Large hyaline	16

Sixteen normoblasts were seen in counting 100 leucocytes. Occasional cells showed polychromatophilia.

The cat was then killed and a post-mortem examination was made. Wasting was marked. The liver, spleen, and intestines were covered with lymph. The liver was definitely enlarged weighing 124 grm., the cat weighing 2,100 grm. A liver from a normal cat weighed 82 grm., the cat weighing 3 kilos.

'In the liver there were grey cellular thrombi in the hepatic veins. Close to the portal sheaths were some areas of haemorrhage, sometimes containing necrosed hepatic cells; these may have been the result of the venous thrombosis. Leucocytosis was very conspicuous throughout the liver. The large veins of the spleen were also thrombosed. The pulp was hyperplastic and engorged. There was no proof of myeloid transformation.'—H.M.T.

The shaft of the femur was well filled with red marrow. Films from this showed almost entirely mononuclear cells of lymphoid and endothelial types, scanty eosinophiles mainly mononuclear, numerous normoblasts and occasional megaloblasts. No phagocytosis was seen.

The changes in the blood as shown by the rapid loss of red cells without marked change in their morphology, and the extreme leucopenia together with the almost total disappearance of the granular marrow cells are identical with the changes in the human cases.

Another cat, which was injected with γ -T.N.T. in which the blood was periodically examined over a period of four to five months, showed no blood changes whatever.

The Liver changes.

Brief details of the experiments.

Cat No. I. 50 mg. doses of α -T.N.T. by subcutaneous injection over a period of thirty-nine days.

Total amount given —350 mg.

Weight on March 12—3,980 grm. ; on April 9—1,040 grm.

Blood examinations.	Mar. 1.	Mar. 11.	Mar. 19.
Haemoglobin		25 %	85 %
Red cells	8,725,000	10,937,000	8,875,000
White cells	21,640	32,000	28,000

Differential :

Polynuclear neutrophils	83.5 %	75.5 %
Coarsely granular oxyphils	5.5	5
Small lymphocytes	7	13
Large lymphocytes	2	4
Large hyaline	2	2.5

Cat died. Post-mortem showed slight cyanosis. An extensive subcutaneous abscess. Viscera were entirely normal in appearance. Sections of the kidney were normal with the normal amount of fat. Sections of *the liver* : 'The central zones of the hepatic lobules were slightly fatty. Leucocytic infiltration of portal sheaths was conspicuous. There was a focal necrosis infiltrated by leucocytes close to a hepatic vein.'—H.M.T.

Cat No. II. 50 mg. doses of α -T.N.T. for twelve days. Total amount 300 mg. Only one blood examination was made which was fairly normal. The cat developed an extensive abscess and was killed. At post-mortem examination there was found to be an extensive subcutaneous slough over the back extending to the abdominal wall. The viscera were normal in appearance. 'There was a hyperplasia of the splenic pulp in which megakaryocytes were present. There was no leucocytosis in the capillaries of the liver.'—H.M.T.

Cat No. III was given a total of 3,125 grm. β -T.N.T., beginning with 50 mg., a dose gradually increased to 125 mg. Over a period of three and a half months. It steadily decreased in weight from 5,340 grm. on March 12 to 1,930 grm. on July 7. The blood serum examined on several occasions was distinctly yellow. Seven blood examinations were made between March 11 and July 7. These counts varied slightly from time to time but within normal limits. The last count before death showed a distinct rise in the total number of white cells from an average about 12,000 to 65,000, and the differential count showed an increase in polynuclear neutrophils from an average of 75 to 97 per cent.

The cat died and post-mortem examination showed wasting. The skin and subcutaneous tissue were distinctly yellow. The liver was large and pale with a yellowish tinge, the lobules were well marked, with light centres, and there were what appeared to be numerous small dilated capillaries on the surface. Kidneys were normal. The spleen was large, firm, and somewhat nodular. Nodules were present in the lung.

Microscopical examination of sections : 'The hepatic cells were fatty, especially in the peripheral zones of the lobules. The only pathological conditions of which I could be certain were a great engorgement with dilatation of many hepatic veins and their tributaries, and a striking number of large mononuclear cells, probably large mononuclear leucocytes, within these vessels. There was con-

siderable hyperplasia of the cells of the splenic pulp, amongst which polymorphonuclear leucocytes were numerous, but myeloid transformation was not detectable. The lung contained parasites, apparently nematodes developing from morulae.'—H.M.T.

Cat No. IV was given 50 mg. β -T.N.T. a day, increased to 100 mg. during the last week for 10 weeks, at the end of that time one blood examination was made, which was entirely normal. The cat died suddenly. Post-mortem examination showed many adhesions about the abdominal viscera, the liver being covered by organizing lymph. About the spleen and pancreas was fairly dense new connective tissue.

On microscopical examination, the liver which weighed 84 grm. showed 'considerable dissociation of the hepatic cells but no degeneration. The dissociation was apparently due to mechanical injury after death. The splenic pulp contained few cells. There was a little recent broncho-pneumonia of the lungs.'—H.M.T.

Cat No. V was given one dose of 250 mg. β -T.N.T. No blood examination was made. Post-mortem: 'Both liver and kidney were engorged. The cells of the central and intermediate zones of the hepatic lobules were fatty. There was a little infiltration of the portal sheaths. There was a vesicular degeneration of the hepatic cells immediately round the portal sheaths.'—H.M.T.

Cat No. VI was given a total of 3.4 grm. of γ -T.N.T. over a period of three and a half months. It decreased in weight from 5,340 grm. on March 12 to 3,000 grm. on July 10. Seven blood examinations were made, all of which were comparatively normal. At post-mortem it showed marked yellow staining of the skin and subcutaneous tissue. Liver was large, dark red, with the lobulation well marked on the surface with blood-vessels forming the periphery of the lobules and light yellow centres with a pin-point red dot in the centre. Kidneys and spleen were of normal appearance. Nodules were present in the lungs.

Sections. 'There was extreme fatty infiltration of the liver. There was no hyperplasia of the splenic pulp. Parasites similar to those in Cat III were found developing in the lung.'—H.M.T.

A kitten which weighed 1,075 grm. was given by Dr. Dale a total of 1,345 mg. of γ -T.N.T. At the time of its death it weighed 650 grm. At post-mortem examination the gall-bladder was found full of pus.

Sections. 'The hepatic cells were dissociated, rounded, sharply stained and free from degeneration. This may represent replacement by regeneration of gradually destroyed cells. I could, however, find no karyokinetic figures. There were several focal necroses infiltrated by leucocytes.'—H.M.T.

Two other kittens were also treated but developed no changes.

In reference to the histological specimens considered above, Dr. Turnbull made the following remarks: 'In the sections from this series of experimental cats I have, therefore, not been able to detect lesions of significance. Focal necroses were present in the liver in three cases, but these necroses resembled those found so frequently at necropsy in man and cannot be taken as examples of the hepatic lesions found in trinitrotoluene workers. In man they are associated with various pathological conditions of the intestinal tract, for instance catarrhal inflammation. The staining of the sections did not permit identification of myeloid transformation, except by the recognition of megakaryocytes. In one case only (Cat No. II) were megakaryocytes present in the spleen, indicating a myeloid reaction such as was found in the two cases to be subsequently described (Cats VII and VIII).'

Cat No. VII was given a total of 2.2 grm. of α -T.N.T. in doses beginning with 50 mg., increased gradually to 200 mg. In addition seven intravenous injections of 2,000 million dead *B. coli* each were given. The experiment lasted seven months, at the conclusion of which the animal died suddenly. The animal lost some weight from 1,850 grm. on May 10 to 1,540 grm. on November 29. Three blood examinations were made, all of which were fairly normal.

On the post-mortem examination Dr. Turnbull made the following report :

'The *liver* weighed 90 grammes. A lobular pattern was shown by red dots separated by a coffee-brown reticulum. In sections stained with Sudan no fat was revealed. There was no evidence of degeneration or disturbance of the normal pattern. The Prussian blue reaction showed numerous Kupffer endothelial cells loaded with iron granules, but demonstrated no iron within the columns of hepatic cells. Certain yellow and grey cylinders seen in the upper border of the left lobes, and suspected at the naked-eye examination to be parasitic, proved to be large hepatic veins distended by thrombi. There was no leucocytosis in the blood of this or of any of the other organs. The *myocardium* (heart, 10 grammes) was free from fatty degeneration. The *kidneys* (together 25 grammes) showed great fatty infiltration of the first convoluted tubules. In the *spleen* (2.5 grammes) the pulp was scanty. A few phagocytes contained ingested red corpuscles, and a very few cells contained granules of iron. Leucocytes were few in the pulp. Several megakaryocytes were present in the organ, one being within an intra-trabecular vein, but no other proof of myeloid transformation was found in sections treated with Jenner's stain. The *marrow of the femur* was mahogany red throughout. The microscope showed small patches of lipomatous marrow, and isolated fat cells scattered through the haemopoietic elements. In the latter megakaryocytes were abundant, myeloblasts were in excess of myelocytes and there were numerous foci of erythroblastic activity. In the *lungs* slight collapse and catarrhal pneumonia in a few subcapsular alveoli were revealed by the microscope. The bronchi and bronchioles appeared normal. Several megakaryocytes were present in capillaries. In the *suprarenal* bodies no abnormality was detected. The *pancreas* weighed 6 grammes. In the subcutaneous tissue of the back were two drachms of pale yellow creamy fluid (injections) in films of which no pus cells nor organisms were found. The inner surface of the skin was tinted yellowish-brown, and the saline-formaldehyde in which the portions of the tissue were fixed was stained next morning the same colour.'

Cat No. VIII was given in all 2 grm. *a-T.N.T.*, beginning with 50 mg. doses increased to 200 mg. Also six subcutaneous injections of 2,000 million dead *B. coli* each were given. The experiment lasted six months. Loss of weight was from 2,240 grm. on June 7 to 1,490 grm. on December 9, when death occurred. Three blood examinations were made, all of which were normal.

Post-mortem report by Dr. Turnbull :

'The *liver* weighed 70 grammes. There was a lobular pattern, small polygonal yellow areas being separated by reddish brown lines. Microscopic examination showed that in the central half to two-thirds of the lobules, and frequently in strands uniting the centres of neighbouring lobules through points equidistant from adjacent portal systems, the hepatic cells were swollen, and often dissociated. Their cytoplasm was relatively faintly stained and eosinophil; it was usually vacuolated, rendered foam-like by numerous vacuoles or occupied by a single large vacuole; occasionally it was hyaline. In Sudan preparations the vacuoles were shown to contain fat. The majority of the nuclei were absent; those that remained were either distorted, or lobed, or pyknotic, or, less commonly, broken into beads (karyorrhexis) or very faintly stained (karyolysis). Into these degenerate and necrosed cell columns there was a slight emigration of polymorphonuclear leucocytes. The Prussian blue reaction with steaming hydrochloric acid revealed abundant iron within the centres of the great majority of the hepatic columns in the healthy peripheral zones, and also in many of the degenerate and necrosed cells of the central zones. Iron was also present in, relatively few, endothelial cells of Kupffer. There was a distinct leucocytosis in the blood throughout the organ, but more conspicuous in the centres of the lobules. White, cellular thrombi were present in many portal veins, hepatic arteries, and hepatic veins. The *myocardium* (heart: 14 grammes) was free from fatty degeneration. The vessels contained a relatively large number of white cells. In a large white *thrombus* resting upon the endocardial surface of the left ventricle the majority of the cells were neutrophil leucocytes, but a few myeloblasts and myelocytes were found (Jenner's stain). In the *kidneys* (together 24 grammes) the first convoluted tubules were loaded with fat, but there were few degenerate nuclei, the condition being, apparently, a fatty infiltration rather than a fatty degeneration. In many ascending limbs of the loops of Henle, however, there were albuminous degeneration and necrosis, whilst there were

many hyaline and granular casts, especially in the descending limbs of the loops of Henle. Thrombi, containing many white cells, were present in several veins. The *spleen* (10 grammes) contrasted sharply with that of the first case. The increase in size and weight was due to a great cellularity of the pulp. The pulp contained a very large number of myeloblasts, many containing karyokinetic figures, a large number of leucocytes, many megakaryocytes, and a considerable number of erythroblasts; myelocytes were, however, very scanty. Many myeloblasts were present in large veins. Many phagocytes contained red corpuscles, and there was a very large number of phagocytes containing coarse granules of iron. The *marrow* of the femur was soft, moist, and a very pale pink. It contained no areas of lipomatous tissue, and only a very few, isolated fat cells. It contained numerous megakaryocytes. Myeloblasts were in great preponderance over all other cells; erythroblastic activity was less than in the previous case, and myelocytes were fewer. In the *lungs* were present catarrhal desquamation in a few subpleural alveoli, and albuminous exudate in one section. The capillaries contained many megakaryocytes, and there was one large megakaryocyte in an arteriole. In the *suprarenal* bodies no abnormality was detected. The *pancreas* weighed 5 grammes. The inner surface of the skin was white.'

Remarks by Dr. Turnbull on the two preceding cases :

'I have, unfortunately, no experience of the normal histology of the cat. That it varies in some respects from the human is shown by the condition of the kidneys in all the cases submitted to me for examination. In the cells of the first convoluted tubules of the kidneys an abundant deposit of fat was unaccompanied by evidence of a corresponding degree of degeneration of the nuclei. Fatty infiltration of the kidney, thus, appears to occur in the cat, though not in man. Conditions such as necrosis can only be regarded as abnormal. Changes in the blood-forming organs found in certain cases only, or in greater degree in certain cases, can also be regarded as abnormal, provided the animals compared are of approximately similar age. Without, however, experience of the reactions of the tissues of the cat, it may be erroneous to ascribe to such changes the same significance as when they occur in man.'

'With these reservations, it may be said that in Cat No. VIII the following abnormalities were present: a fatty degeneration and necrosis of the central zones of the hepatic lobules; haemosiderosis of the liver; parenchymatous degeneration of the kidneys; a general leucocytosis and myelaemia; a conspicuous myeloid, chiefly myeloblastic transformation of the splenic pulp; leucocytosis of the spleen; great erythrocytic phagocytosis in the spleen; increased activity of the haemopoietic marrow, in which myeloblastic activity greatly preponderated.'

'There were, therefore, two main lesions—a central degeneration with necrosis of the hepatic lobules, and a disturbance of the blood-forming organs. The degeneration and necrosis of the liver might have been due to severe anaemia, and the myeloblastic activity of the marrow and accessory blood-forming organs might correspond to that which is found in pernicious anaemia in man. Evidence of excessive destruction of red corpuscles was given in the spleen by the phagocytosis of erythrocytes and the great accumulation of coarse granules of iron derived from ingested corpuscles, and in the liver by the extensive haemosiderosis of the hepatic columns. But, severe anaemia appears to be excluded by the absence of fatty degeneration of the myocardium, and by the relatively slight part played by erythroblastic activity in the changes in the blood-forming

organs. In all severe anaemias in man erythroblastic activity is conspicuous ; even in aplastic anaemia, such haemopoietic marrow as is present is almost entirely erythroblastic.'

'The changes in the blood-forming organs resembled those resulting in response to an excessive call for leucocytes, much more closely than those occurring in anaemia. It would appear, therefore, that the disturbance of blood-formation was not essentially anaemic, and was not the cause of the hepatic necrosis. More probably the disturbance of blood formation was instigated directly or indirectly, by agents which caused necrosis in the liver.'

'The tissues in Cat No. VII showed only slight abnormalities when judged by the standard of normal human tissues. They serve, therefore, at any rate as a control to those of Cat No. VIII. The abnormalities were : evidence of myeloid transformation of organs afforded by the presence of megakaryocytes in the splenic pulp and in the vessels of the lung ; abundant iron in the endothelial cells of the liver. If these are not normal occurrences in cats, they indicate a slight degree of the disturbance of the blood-forming organs which was found in Cat No. VIII.'

Cat No. IX was given 13 c.c. of toluol in 5 c.c. doses over a period of three months. This was done as a control in order to note the effect of toluol as, in all the experiments, the T.N.T. was given dissolved in toluol. There was some loss of weight from 1,630 gm. on May 10 to 1,230 gm. on July 9. Two blood examinations were made, both of which were normal. The blood serum was clear white. The post-mortem showed nothing abnormal to the naked eye.

Sections showed: 'The hepatic cells contained much fat, the cells in the central zones containing more than those in the peripheries of the lobules. There was, however, very little nuclear degeneration. A few portal systems were infiltrated. There was one small, focal area of necrosis ; it was partly haemorrhagic and was bordered by a zone of leucocytic infiltration. The splenic pulp was not hyperplastic and contained no megakaryocytes.'

RÉSUMÉ

Rabbits and rats exposed to NO vapour developed cyanosis and methaemoglobinaemia but none of the major changes of T.N.T. poisoning.

Ammonium nitrate given hypodermically to a rabbit over a period of ten weeks had no effect.

Five rabbits injected hypodermically with T.N.T. dissolved in toluol suffered from local necrosis at the site of injection but no other lesion of importance.

An attempt to sensitize a rabbit by alternating small and large doses of T.N.T. failed.

The three T.N.T. isomers were separately injected into cats. The lethal dose of α -T.N.T. was greater than that of β or γ but repeated small doses of α more constantly produced drowsiness, loss of appetite, and loss of weight. Both β - and γ -T.N.T. produced a yellow pigmentation of the blood serum and subcutaneous tissues.

Local necrosis at the sites of injection were set up by all the isomers dissolved in toluol.

The two major lesions of T.N.T. poisoning were approximately produced in two experimental animals only.

A cat given γ -T.N.T. acquired shortly before death the exact and typical blood changes of aplastic anaemia. The rapid alteration in the circulating blood of this animal was most striking and simulated precisely the human cases.

In one cat only were any striking changes induced in the liver, a marked necrosis and fatty degeneration of the central lobules being found post-mortem. This animal received α -T.N.T. together with subcutaneous injections of dead colon bacilli.

VI. EXPERIMENTAL WORK UPON T.N.T.
POISONING AND THE PATHOLOGICAL
CHANGES INDUCED BY T.N.T. IN HUMAN
BEINGS.

BY P. N. PANTON, M.B., B.C., Cantab.

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EXPERIMENTAL.

The problems which in the course of this investigation particularly called for experimental work were in the first place the manner in which the poisonous substance was absorbed—whether by inhalation, ingestion, or through the skin, then the exact nature of the poison and finally the mode by which the toxic effects were produced.

The Mode of Absorption.

The three paths to be considered were inhalation, ingestion, and absorption through the skin, and to any one who has visited a T.N.T. factory the last path is the most obvious. That nitro-bodies such as dinitrobenzene can be absorbed through the skin of cats and human beings had been previously and conclusively proved by White and Hay (1). The proof that T.N.T. is mainly absorbed in this manner was adduced by Dr. Benjamin Moore (2) and his repeated advocacy of skin protection, coupled with more cleanly—and incidentally safer—methods of handling T.N.T. had the greatest influence in restricting the dangerous results of T.N.T. absorption. The experiments on T.N.T. absorption made by Dr. Moore and his co-workers were both animal and human, the latter being entirely convincing both that T.N.T. can be absorbed through the unbroken skin in ordinary manipulation, and that poisonous results can arise from such absorption. The test of absorption was furnished by the appearance of Webster's reaction in the urine and the toxic sequel was manifested in the person of Dr. Moore himself (2).

The amount of T.N.T. that can be ingested by the introduction of dirty fingers into the mouth is evidently small compared to that

constantly rubbed through large exposed areas of skin and the amount of dust that could be swallowed by an operative during a day's work was shown by Dr. Moore, in dust extraction analysis, not to exceed 10 mg.

Similarly the amount possible to be inhaled by those working near molten T.N.T. was shown to be very small, though some of the worst early cases I saw were among this class of operative.

Koelsch (3), while admitting skin absorption, laid stress upon the importance of inhalation, but he was convinced of the non-toxic properties of T.N.T. and later (4) obsessed by the toxicity of tetra-nitromethane which occurs as an impurity.

The problem on the whole seems fairly comparable to the older controversies on lead poisoning. The painter absorbs lead constantly and considerably through the skin, he occasionally adds small quantities of it to the food he takes with his meals, but he may inhale it to a dangerous extent in the process of burning off the paint. Similarly the main path of absorption of T.N.T. is undoubtedly the skin, further small amounts may be accidentally ingested and to a more problematical extent the vapourized substance may be inhaled. It must be admitted, however, that the minimal fatal dose for a human being may be minute. There are very definite instances of major poisoning in which the amount absorbed through the skin must have been infinitesimal and the poisoning presumably resulted from the amount ingested. In view of the minute bulk of T.N.T. which may be fatal to a susceptible person, all channels of absorption are important, even though the main channel for the majority is the skin.

The Exact Nature of the Toxic Substance.

Here again the most obvious, though not necessary correct, solution is that the actual poisonous substance is T.N.T., since T.N.T. forms the great bulk of the explosive powder even in its commercial state. T.N.T. had, however, primarily been considered to be non-toxic in marked contrast to dinitrobenzene, and certain other impurities and decomposition products of T.N.T. had to be considered.

A possible source of poisoning to which attention was called in an early stage of the inquiry was the inhalation of fumes given off by T.N.T. Nitrous fumes in amount detectable by the sense of smell are given off by T.N.T., and the toxic effect of T.N.T. was ascribed to these fumes by, among others, the managing director and staff of the National Shell Filling Factory at Chilwell. As has been pointed out by Dr. Moore (10, p. 70) workers in processes involving the inhalation of much larger quantities of nitrous fumes than ever obtained in T.N.T. filling are not affected by either jaundice or anaemia, and the nitrous fumes extracted by him from the neighbourhood of molten T.N.T. were infinitesimal. Rabbits and white rats were exposed by me to a percentage of nitrous fumes in a fume chamber greater by far than ever could occur in any T.N.T. process, and though it was found that the animals, even with small quantities of gas, showed cyanosis and gave a positive Haldane test for methaemoglobinaemia, neither jaundice nor anaemia was pro-

duced in any instance. The exposures of these animals to NO fumes extended over 3 months, each exposure lasting about 3 hours. In spite of the expressed opinion of the staff at Chilwell, the danger of nitrous fumes in T.N.T. filling processes can, I think, be disregarded as a cause of major poisoning.

Another T.N.T. impurity to receive attention, both in this country and in Germany was tetranitromethane. Koelsch (3) considers the severe toxic effects to be due to impurities, of which he mentions nitrated benzols and non-nitrated hydrocarbons, remains of nitrous fumes, and tetranitromethane. The action of $C(NO_2)_4$ is, however, a direct one on the respiratory passages, and Koelsch failed to obtain any evidence of its action except as a possible cause of some of the minor forms of T.N.T. poisoning, including cyanosis. There is no evidence or suggestion that either toxic jaundice or aplastic anaemia can be produced by this substance, and the amount of tetranitromethane obtained in samples of T.N.T. in this country was small. Similarly the benzols occur in very small amount only. The only substance used in bulk with T.N.T. is the ammonium nitrate which is mixed with T.N.T. in amounts up to 80 per cent., but this substance is commonly held to be quite innocuous. A rabbit given by me $\frac{1}{2}$ -gram. doses daily (with certain intervals) under the skin for 3 months was quite unaffected.

By a process of elimination the conclusion is reached that the poisonous substance is T.N.T., but certain difficulties obstruct the direct proof. The main difficulty in the first place was the original failure to reproduce the major lesions of T.N.T. poisoning in animals, and this has only been partially surmounted. Further it has been difficult to explain the rarity of the lesions among the large numbers handling the powder. One would like also to account for the strange latency, often enduring months, before symptoms arise. Before describing the nature of the animal experiments performed the possible explanations to which they were directed may be mentioned.

The metabolism of the drug within the body may be different in different species of animals. It has long been known that toluylenediamine affected dogs quite differently to guinea-pigs.

Explanation was required for the low rate of incidence and the fact that the workers affected became so within a few weeks of commencing work with the powder, severe poisoning among those who had been at work for many months being practically unknown. The usually accepted explanation is that of individual susceptibility, though the phrase can hardly stand for an explanation, and the obvious analogy is the prevalence of alcohol drinking and the comparative rarity of alcoholic cirrhosis of the liver. Various possibilities suggested themselves, and among them that poisoning might arise by a species of sensitization by which operatives taking small doses of poison at intervals might render themselves sensitive to a subsequent dose; such an explanation would be most unfavourable to the generally adopted system of alternation of labour, and in fact alternation did not increase poisoning in factories. Another suggestion was that the susceptibility of the individual depended upon a second factor, such a factor being a possible infection of the biliary passages by a coliform bacillus rendering the liver more

susceptible to the action of the T.N.T. Further factors were differences in the permeability of the skin of individuals and individual differences in the metabolic processes by which the drug was dealt with. Another possibility was that of variation in the composition of the drug itself, and attention was here directed to the separate toxic effects of the three T.N.T. isomers. The results of the various experiments made will be briefly recorded.

That the metabolism of the drug varies in different species of animals was proved by Mr. Webster and Dr. Wyon, working under the auspices of Dr. Dale and Dr. Barger. The animals used in the early experiments were rabbits, guinea-pigs, and rats, and it was subsequently shown that cats are very much more sensitive to T.N.T. than rabbits. It was found that rabbits taking T.N.T. excreted in the urine a chromogen giving a reaction identical with that found in man, but that this substance is not present in the urine of the more susceptible cat. The difference in susceptibility of the two species of animals helped to explain the difficulty in reproducing the lesions of T.N.T. poisoning in the rabbit. Since man stands in an intermediate position between herbivores and carnivores it is conceivable that individual differences in the diet and metabolism of man might produce different susceptibilities to the drug.

Dr. Moore found Webster's test to be positive in the urines of all those absorbing T.N.T. but to a different degree in different individuals. He further found different degrees of permeability of the skin, and it is possible that some cases of poisoning arose by an overdose owing to an abnormal permeability of the skin of the individual.

Attempts by Dr. Vaidya and myself to sensitize rabbits by alternating small and large doses of T.N.T. (from 100 to 750 mg. given hypodermically) at intervals of a week to ten days failed to have any other effect than the more regular mode of administration.

The suggestion of Dr. Laidlaw that the introduction of colon bacilli in addition to the T.N.T. might succeed in producing the liver changes was tried by Dr. Bates and myself. Two cats were injected over periods of six months with T.N.T. α , and at the same time were given large doses of *B. coli*. In one cat some of the bacterial injections were made intravenously with live bacilli, and in the other cat hypodermic injections of dead organisms were given. Changes were produced in the liver of one of these cats and are referred to later.

The different effects of the 3 T.N.T. isomers were investigated by Dr. Bates and myself with the advice and assistance of Dr. Dale. The great bulk of the T.N.T. powder used consisted of the symmetrical T.N.T. α . The β and γ -T.N.T.s vary in amount from a mere trace to 4 per cent. in an exceptional sample analysed by Mr. Tutin. If it could have been proved that the β and γ substances produced in cats lesions of toxic jaundice or of aplastic anaemia whereas the α -T.N.T. did not, the problem of the precise nature of T.N.T. toxicity would have been a little nearer to solution. The reproduction of the human lesions in animals has turned out to be extremely difficult. Dr. Moore, in a large number of experiments, mainly with rabbits, guinea-pigs, and rats failed to reproduce the liver and blood changes except in one possible instance

when certain liver changes were found.¹ Dr. Vaidya and myself similarly failed to reproduce the lesions in rabbits which appear particularly resistant to the drug. Cats were known to be more susceptible and it was found possible to produce some liver changes, and in one cat typical central necrosis of the liver lobules, though never the massive necrotic action of the human cases, and one typical example of aplastic anaemia was developed. The results of the experiments will be given very briefly, but it must be recognized that the number of animals experimented upon were wholly inadequate for conclusive results. The amount of work involved by even a small number of experiments was more than could be accomplished in the time at our disposal.

Thirteen cats were injected subcutaneously with T.N.T., and since the T.N.T. powder was dissolved in toluol, 1 cat was injected with toluol only as a control. It was particularly attempted to reproduce either aplastic anaemia or toxic jaundice or both.

The blood condition may be considered first. In 8 cats periodic blood examinations were made, and in 7 no specific blood changes were produced. The eighth case is of considerable interest. A cat had been given for from 9 to 10 weeks small doses of γ -T.N.T. by Dr. Dale. The blood was examined and the following result found:

Red cells	6,750,000 per c.mm.
White cells	22,800 per c.mm.

Differential count :

Polynuclear neutrophils	89.5 %
Small lymphocytes	5.0
Large lymphocytes	0.5
Large hyaline	5.0

One normoblast was seen among 200 leucocytes and some polychromatohhilia.

A comparatively normal blood count for a cat.

Six days later the cat was very ill, and the following striking change in its blood condition was found to have occurred :

Red cells	1,937,500 per c.mm.
White cells	600 per c.mm.

Differential count :

Polynuclear neutrophils	8 %
Small lymphocytes	58
Large lymphocytes	18
Large hyaline	16

Sixteen normoblasts were seen in 100 leucocytes. Occasional cells showed polychromatophilia.

The cat was killed soon after this blood count was done and the shaft of the femur was found well filled with red marrow. Films showed almost entirely mononuclear cells of lymphoid and endothelial types, scanty eosinophiles mainly mononuclear, numerous normoblasts and occasional megaloblasts.

The changes in the blood as shown by the rapid loss of red cells without marked change in their morphology, and the extreme

¹ These liver changes occurred in a rabbit and were identical with those produced in one of our cats. I am unable, however, to find any record of the experiment from which the liver specimen was obtained.

leucopenia, together with the almost total disappearance of the granular marrow cells, are identical with the changes in the human cases.

On the other hand a cat injected with γ -T.N.T. by Dr. Bates and myself, in which the blood was periodically examined over a period of 4 to 5 months, showed no blood changes whatever during life. These two cats were the only animals treated with γ -T.N.T. in which the blood was examined.

No blood changes were found in the cats treated with T.N.T. α or T.N.T. α and *B. coli*, T.N.T. β and toluol.

The liver changes. The histology of the tissues examined was in the main referred to Dr. H. M. Turnbull, and is reported at greater length by Dr. Bates and myself elsewhere. In the rabbits and rats examined no liver changes of note were produced. In six cats and three kittens injected with T.N.T. α , β , and γ , and in a control cat injected with toluol no hepatic lesions were produced which could be certainly ascribed to the drugs given, and in only one case was any myeloid reaction found in the tissues.

Two other cats were given T.N.T. α and *B. coli* in addition, and in one of these definite liver changes were produced. The changes consisted in a fatty degeneration and necrosis of the central zones of the hepatic lobules, haemosiderosis of the liver and a myeloblastic activity of the marrow and accessory blood-forming organs. The hepatic changes were considered to be due to the toxic agents employed and to be independent of such anaemia as might have resulted from the disturbances of blood formation. The second cat showed myeloblastic activity but no liver changes of importance. The hepatic lesions in the former case were very similar to those produced in a rabbit by Dr. Moore and the results of these few experiments in which bacterial suspensions were added to the T.N.T. injections, do not in reality lend much weight to the views that a second factor is necessary to produce the lesions, and that the factor is a bacterial toxin.

The effect upon the cats of these drugs, other than the histological changes in the blood and viscera, may be briefly mentioned. Loss of weight was a constant feature and was well marked. One cat, treated with β -T.N.T. over 3 months, during which time it received in all 3,125 grm., went from 5,340 grm. to 1,930 grm. On the other hand the control cat injected with toluol lost weight appreciably.

Cyanosis, as evidenced by duskiness of the gums, was noticeable in the early stages of chronic poisoning, but later the cats became apparently jaundiced, and the cyanosis less obvious.

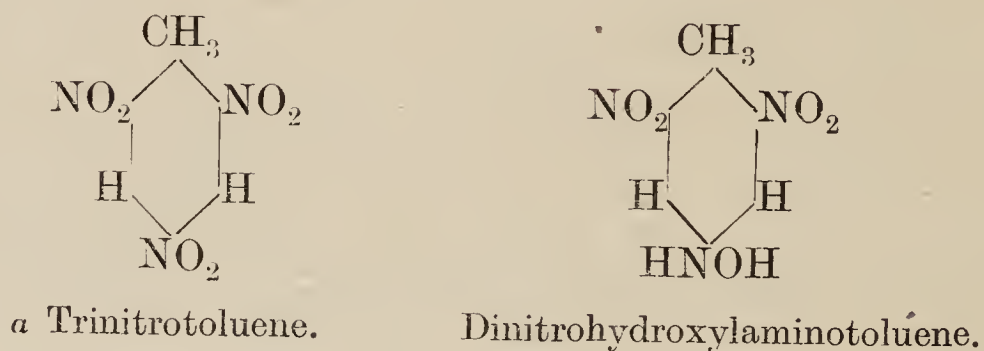
Jaundice, as evidenced by a yellow colour of the mouth and of the blood serum, was constantly present but in varying degree. The serum of a kitten, injected by Dr. Dale, was of a bright yellow colour, and the serum of our animals when examined was definitely yellow when compared to the white serum of a normal cat or of the cat receiving toluol. The skin and subcutaneous tissues post-mortem were sometimes noted to have been deeply yellow. The yellow colour, however, was not due to the presence of bile pigments in the blood but to a combination of the T.N.T. with the body proteins producing a bright yellow reaction. The yellow colour:

of the blood serum was particularly marked in all the β and γ T.N.T. cases and was not noted in any of the cats treated with pure α T.N.T. Local necroses at the site of inoculation of the drugs were extremely common with all the isomers. These necroses were usually aseptic but occasional considerable abscesses developed, and the animal had to be killed.

The effect upon the general condition of the animals was, in the case of small repeated doses, e. g. from 50 mg. upwards, to produce torpidity and listlessness, and unless frequent intermissions in the treatment were made the cats became drowsy, went off their food, lost weight rapidly and died. The necessity for intermissions was particularly noticed in the cats treated with T.N.T. α . Although the lethal dose for the cat of T.N.T. β or γ is smaller than that of α , repeated small doses are worse tolerated in the case of α .

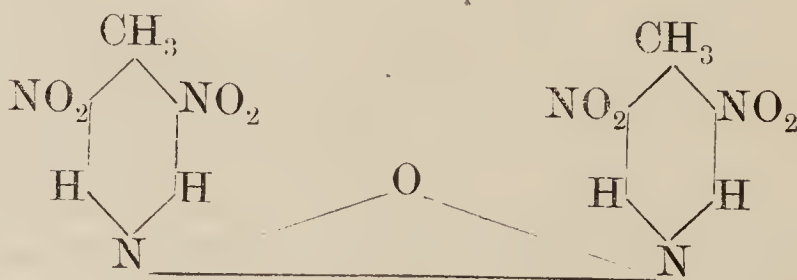
The metabolism of T.N.T. within the bodies of man and animals has been already referred to. The following brief account is taken from the work of Webster and Wyon, investigating under the supervision of Drs. Dale and Barger, and the conclusions given in their report have been revised from subsequent information given me by Dr. Dale.

After absorption through the skin T.N.T. is possibly reduced to dinitrohydroxylaminotoluene.



The latter substance has not, however, been identified in the urine, but appears as an azoxy compound which can be formed by condensation of dinitrohydroxylaminotoluene by an acid or alkali.

The azoxy compound has the formula



and is excreted in the urine in conjugation with glycuronic acid.

The azoxy compound is the substance which gives Webster's test. In animals, however, who are given T.N.T. in massive doses, only a small portion of the T.N.T. excreted appears in this form, the bulk of the excreted derivative has a different composition, the nature of which is discussed elsewhere in this report, and does not give Webster's test.

The azoxy compound has been isolated from the urines of T.N.T. workers, of rabbits, and of a monkey. It is absent from the urines of cats.

In the free condition the azoxy compound has about 3 times the toxicity of the original T.N.T.

The lethal dose of T.N.T. for a cat is about 0.25 grm. per kilogram body weight, whether given hypodermically or by the mouth.

The different modes of metabolism of T.N.T. in man, the rabbit, and the cat, are worth considering further in conjunction with our clinical knowledge of the effects of T.N.T. on man.

Webster's test for the T.N.T. derivative in the urine was originally considered by Dr. Moore to be a test for the susceptibility of the individual. Those operatives giving an intense urinary reaction would have been, on this assumption, refused T.N.T. work. Subsequent investigation renders the opposite of this hypothesis more probably correct. The great majority of human beings metabolize T.N.T. in a similar mode to the rabbit. Both man and rabbit are comparatively immune to the poison. Is it not possible that the very exceptional man metabolizes T.N.T. in an exceptional manner? Unfortunately we have no records of operatives who failed to give the T.N.T. reaction in the urine and who subsequently proved susceptible, but there is some clinical evidence in support of the theory.

Dr. Moore found a positive Webster's test in the urine up to two weeks after contact with T.N.T., yet in not a single case of toxic jaundice has the test been found positive in the urine, and numerous cases have been examined in this respect within two days of leaving work. Further, in one case of severe toxic jaundice, which subsequently recovered, T.N.T. was rubbed into the skin, and Webster's test continued to be negative in the urine.

The evidence is certainly not complete and is open to criticism. Dr. Castellain has informed me of 14 operatives in liquid T.N.T. whose arms were covered in the T.N.T. solutions, none of whom gave Webster's test and all of whom remained healthy; but poisoning from this particular branch of T.N.T. work appears to be rare, and it is conceivable that T.N.T. dissolved in paraffin is less readily absorbed by the skin. Dr. Castellain further suggested the possibility that bile in the urine might interfere with Webster's test, and there is the further possibility that the disorganization of the liver interferes with the normal metabolism.

On the whole the theory of abnormal individual metabolism seems to have more bearing upon the explanation of individual susceptibility than any question of abnormal variation in the powder either from the presence of impurities or the particular action of isomers.

PATHOLOGICAL.

The path by which T.N.T. is absorbed, the more concise account of its mode of metabolism in the bodies of man and animals, and the exact nature of the poison which produces the visceral disturbances to be described are more conveniently discussed in the experimental section. It may be briefly stated here that the main channel of absorption is the skin, and that the actual poisonous substance is almost certainly T.N.T. itself.¹

¹ T.N.T. is here meant to include the symmetrical T.N.T. which forms the great bulk of the powder and the isomers β and γ T.N.T. which, in an exceptional sample examined by Dr. Dale, constituted as much as 4 per cent. of the powder.

The pathological effects of T.N.T. absorption by the human subject are evidenced locally : in the process of elimination of the substance, and by its action on certain internal organs, particularly the liver and bone-marrow.

The local action of T.N.T. produces universally a yellow discoloration of the parts of the body exposed to the powder. In susceptible individuals a localized dermatitis is set up in addition to the discoloration. Further Dr. W. J. O'Donovan has informed me of three fatal cases of Ludwig's angina occurring among T.N.T. workers, in all of whom there was severe oral sepsis, and it is possible that the local action of T.N.T. powder upon the mouth affected the original course of the disease. The local necrosing action of T.N.T. is frequently seen in experiments on animals and is referred to later.

In the process of elimination certain symptoms and physical signs dealt with in the clinical section of the report may be set up. Of these, gastritis may be due in some cases to the irritant action of the T.N.T. directly absorbed from the mouth into the stomach, but probably the more usual path to the stomach is by way of the skin. The drug is partly excreted by the skin, and a red staining of the clothes in the axillary region is not uncommon, the red colour following the addition of alkali when the clothes are washed. Rabbits taking T.N.T. excrete large amounts of a bright pink pigment which collects upon and adheres to the fur. The faeces of operatives in T.N.T. factories do not appear to have been systematically searched for T.N.T., but the unchanged substance has been detected by Dr. Moore (2) in the faeces of rabbits which had received large doses by the mouth. The unchanged substance cannot be detected in the faeces or viscera of persons recently employed on T.N.T.

The main channel of elimination is the urine. T.N.T. is not excreted as such in the urine since extraction by ether fails to remove it. The trinitrotoluene after preliminary reduction of one of the nitro groups is excreted in the urine in conjugation with glycuronic acid. The reduced T.N.T. can be liberated from the glycuronic acid by treatment with an acid, and a colour reaction has been devised for its detection. This test for the detection of T.N.T. as it is eliminated in the urine was worked out by Mr. T. A. Webster (5), and is performed as follows :

Measure out $12\frac{1}{2}$ c.cm. of the urine in a measuring cylinder, then add $12\frac{1}{2}$ c.cm. of diluted sulphuric acid, made up by mixing 20 c.cm. of strong sulphuric acid with 80 c.cm. of water. Pour the mixture of urine and acid into a separating funnel of 100 to 150 c.cm. capacity and provided with a stopcock ; add to the mixture 10 c.cm. of ethylic ether (the ordinary ether made from methylated spirit is sufficiently pure for the purpose), shake up well and allow to settle ; take out the cork or stopper from the top of the separating funnel, open the stopcock at the bottom and allow the mixture of acid and urine to run off, then turn the stopcock off so as to retain the ethereal solution in the separating funnel. Now add 25 c.cm. of tap water to the ethereal solution in the separating funnel and shake up again to remove the traces of the mixture of urine and acid, and allow to settle again for two or three minutes, then run off the water by opening the stopcock, retaining the ether in the funnel. Finally, let the ethereal solution flow into an ordinary test-tube and try for the presence of T.N.T. in it as follows :

Prepare a solution of alcoholic potash by dissolving 4 to 5 grams of caustic potash in 100 c.cm. of methylated spirit or absolute alcohol. Where many tests are to be carried out this solution may be made by having a stock saturated

solution of caustic potash, and adding, when a fresh quantity of the reagent is required, 10 c.cm. of this to 90 c.cm. of alcohol.

To the ethereal solution obtained as above described 5 c.cm. of this alcoholic solution of potash are added. When T.N.T. is present a purple coloration is at once developed, varying in intensity according to the amount of T.N.T. present, from the faintest trace to a deep purple. The colour changes rapidly from the purple to a brown colour, and it has been found that the best results as to intensity are obtained by judging rapidly after the colour is struck.

Webster's test was subsequently shown by Frank Tutin (6) to have a source of error since various purgatives, including rhubarb, senna, and cascara contain anthraquinone derivatives which give a colour test very similar to that produced by T.N.T. Tutin devised the following modification which excludes anthraquinone derivatives :

The urine should first be extracted with two successive portions of ether. This treatment removes any T.N.T. which may be present from accidental contamination, and also chrysophanol. The urine is then acidified with hydrochloric or sulphuric acid and again extracted with ether. During these extractions the addition of alcohol is usually necessary to cause the separation of emulsions. The ethereal extract of the acidified urine must then be extracted twice with dilute aqueous sodium carbonate, washed with water, and then treated with alcoholic potash, when the characteristic violet colour will be produced if derivatives of T.N.T. were present in the urine. If anthraquinone derivatives were present the sodium carbonate extracts will have a reddish-violet colour.

The toxic actions of the major kind by T.N.T. upon the internal organs are rare. The actual incidence is dealt with elsewhere, but it may be mentioned here that with exceptional opportunities, and in the space of about one year, I saw 30 examples only. The rarity of these serious effects led to the pre-war belief that T.N.T. was devoid of serious poisonous properties. The explanation of the rarity will be discussed later. The main toxic action of T.N.T., other than the production of minor ailments such as gastritis and dermatitis, is chiefly manifested upon the liver and the blood-forming organs.

The changes produced in the blood and blood-forming organs may be summarized from papers by Dr. Matthew Stewart (7 and 10) and myself (9 and 8).

In 50 women at work and free from symptoms no changes in the red cells nor in the haemoglobin percentage were found. The only change found in the blood was a moderate leucocytosis, giving an average of 10,000 leucocytes per c.mm., the highest total counts showing a relative increase in the polynuclear neutrophils. The deductions drawn were that a daily absorption of T.N.T. by the majority of individuals has no destructive action upon the blood, and has a stimulating action upon the granular leucocytic tissue of the bone-marrow.

Cyanosis was found to be common. No fewer than 78 out of 100 women examined on a cold day showed some degree of cyanosis, and of these 18 were strikingly cyanosed. The cyanosis follows the absorption of T.N.T. and is due to a mixture of NO haemoglobin and methaemoglobin in the circulating blood. Marked cyanosis may be present without symptoms, and a serious degree of methaemoglobin-aemia does not seem to occur. Koelsch (3) failed to produce methaemoglobinaemia in animals, and concluded that cyanosis and other

symptoms of poisoning did not occur with pure T.N.T., but were due to impurities in the war product. These views will be again referred to later. Dr. Moore (2), one of the first to call attention to the frequency of cyanosis in T.N.T. workers, assumes that the methaemoglobin-carrying red cells are broken up, and that a compensatory increased production of new cells puts a strain upon the red marrow. He follows out his assumption by ascribing aplastic anaemia to exhaustion of the red marrow following this blood destruction and toxic jaundice to liver exhaustion following upon the excessive haemoglobin metabolism. Dr. Moore's deductions are accompanied in his paper by arguments which appear to me fallacious, and his original assumption of constant blood-destruction is unsupported by any proof and directly opposed to available evidence. Large quantities of blood withdrawn at regulated intervals may subsequently lead to an erythraemia, though I have not found this to occur among 'donors' used for repeated transfusions of blood, but continuous abnormal blood destruction in human pathology, including destruction consequent upon malarial infection, invariably leads to anaemia, and these cyanosed workers are neither anaemic nor polycythaemic. Also T.N.T. anaemia, when it does occur, is of a rapidly fatal kind, accounted for by destruction of the bone marrow rather than the blood. Methaemoglobinaemia may be pushed to a marked degree without producing any apparent red cell destruction. From the experiments of Haldane, Makgill, and Mavrogordato (21) it is quite clear that mice rendered methaemoglobinaemic to an extreme degree (by hypodermic injection of sodium nitrite 74 per cent. of the haemoglobin was converted into methaemoglobin) and subsequently recovered by exposure to oxygen, must have exhibited the property of the red cell to recover from methaemoglobin combination without permanent injury. The freedom of aniline workers from toxic jaundice is also noteworthy.

Cholaemia. Twenty per cent. of workers showed slightly tinged sera, a very frequent phenomenon among normal people; 3 per cent. only showed sera at all deeply tinged with bile, and the conclusions drawn were that a bile-tinged serum was insufficient evidence of T.N.T. poisoning, and that no proof was obtained of any constant minor action of T.N.T. upon the liver of the average worker.

Toxic jaundice. In 24 cases of toxic jaundice, as well as in certain other examples of minor T.N.T. sickness, the blood was entirely normal, except for the presence of bile pigment in the sera. There was no increased fragility of the red cells. In one severe and seven convalescent cases the Wassermann Reaction was negative. Dr. Jacob, in Nottingham, found the Wassermann Reaction positive in three consecutive cases, and the probability was that these three cases were syphilitic also. In the cases that recovered, bile pigment was commonly found to persist in the serum for many months. The conclusion arrived at was that the jaundice was not haemolytic in origin but was a toxic jaundice consequent upon the liver destruction.

Aplastic anaemia is the rarest and most fatal of the T.N.T. complications; it is doubtful if recovery ever occurs. It is characterized during life by a primary anaemia with a high colour index, rapidly progressing to an extreme degree. The red cells are unaltered

morphologically, the granular white cells are diminished until a total leucocytic count of 1,000 per c.mm. may be reached, of which the polynuclear neutrophils form only 4 per cent. The serum is colourless, often in marked contrast to the greenish-yellow serum present in pernicious anaemia. Post-mortem the blood-forming marrow is reduced to an extreme degree.

It would appear that haemolysis occurs, at any rate in the terminal stages, because in the cases in which the aplastic nature of the anaemia was proved, there was a conspicuous haemosiderosis of the liver. In one case, however, in which the anaemia was in all probability aplastic, no haemosiderosis of the liver could be detected. Further, in the proven cases of aplastic anaemia pigment derived from haemoglobin was not demonstrable in the blood during life. The essential lesion is a reduction of the blood-forming marrow. There can be little doubt that this is due to the direct action of the poison upon the blood-forming cells in the marrow. Direct evidence of injury to the marrow cells is afforded by the extensive phagocytosis of nucleated cells found in one of the two cases in which a systematic microscopic examination of the bone-marrow was made. The aplastic anaemia may occur quite independently of the jaundice, though persons susceptible to the toxic action upon the liver are more susceptible to the action upon the marrow also. Thus of 42 cases of toxic jaundice observed by Captain Stewart and myself 7 or 16·6 per cent. developed aplastic anaemia subsequently. The anaemia may, however, arise in the absence of any clinical or histological liver changes. The well-known latent period which may elapse between exposure to T.N.T. and the appearance of jaundice is usually exceeded by the interval before the appearance of anaemia. Consequently in some jaundiced cases it has been possible to watch the subsequent development of the anaemia from the time of the first appearance of any blood changes. Purpura has been a prominent clinical feature of some of the aplastic anaemia cases, and is probably of the same nature as may occur in other severe forms of anaemia, and particularly in the acute leukaemias.

The post-mortem changes. The changes found in the fatal jaundiced cases, together with the post-mortem findings in aplastic anaemia, may be considered together. The histology of these conditions has been worked out by Dr. H. M. Turnbull, and the following description is based upon a paper read by him before the Royal Society of Medicine (10) with certain modifications and additions subsequently communicated to me by him. At the same meeting a communication was also made by Dr. Stewart whose paper is accompanied by illustrative plates. Reference may also be made to the report of a case in the United States by Martland (11), giving a brief histological account of the liver changes in a toxic jaundice case, and to a Canadian case described by Dr. Horst Oertel (12), who concluded that T.N.T. acted, not upon the parenchyma of the liver, but primarily upon the endothelium of the blood-vessels and lymphatics. Dr. Oertel's case does not appear to be a pure case of toxic jaundice, and though no mention of any blood examination is given, the account of the case suggests toxic jaundice, with partial recovery and subsequent death from aplastic anaemia.

The changes described by Dr. Turnbull in 7 cases of severe jaundice are given first.

The Liver was greatly reduced in size. Externally were large areas of depression over which the capsule was slightly thickened and opaque, and projecting areas over which the capsule was transparent and revealed a lobular pattern. The cut surface showed similar raised and depressed areas, some of the former appearing as small projecting nodules. The depressed areas showed grey points, corresponding to portal sheaths, set close together in a firm, red ground. The raised areas were soft, and showed a lobular pattern marked out by dark yellow or bright red centres and bright yellow peripheries; elongation and anastomosis of portal sheaths were often recognizable. The largest area showing a lobular pattern lay, except in one case, in the anterior aspect of the upper part of the right lobe. A great portion, from one-third to two-thirds, of the right lobes was destroyed. The whole left lobes were greatly shrunk and consisted almost entirely of the depressed tissue. The spigelian lobes, with one exception, were completely or almost completely destroyed. The weights of the livers varied from 17 oz. (in a male aged 14) to 31 oz. (in a male aged 35). Microscopically there was degeneration and necrosis of parenchyma associated with infiltration and fibrosis. Destruction of parenchyma was complete in the sunken and incomplete in the raised areas. The process commenced with a degeneration partly fatty, partly dropsical, and partly hyaline, which began round the central and other hepatic veins, and might extend from lobule to lobule at the points farthest from the portal systems. The more severe and acute the degenerations and subsequent necroses were, the less appeared to be the part played by fatty degeneration. In the degenerate areas the capillaries became dilated and small haemorrhages were frequent. A cellular infiltration with polynuclear leucocytes took place, and later a fibrosis accompanied by the cells of chronic inflammation. The final fibrosis in these cases in which the hepatic destruction proved fatal, was delicate. In the depressed areas the hepatic cells in the peripheral zones of the lobules were destroyed as well as those in the centre, and relatively little fat was present. No free iron pigment was demonstrated in any liver. Regeneration in the raised areas was slight or absent, and most evident where the duration of the lesions was longest, as estimated by the density of the fibrosis.

The bile canaliculi were distended by bile, and more obviously in the hepatic columns next the central necrosis than in those near the portal systems. Bile was rarely present in the portal ductules, almost all of which were collapsed. The portal ducts were empty of bile; in one case there was catarrhal desquamation, in another slight infiltration with leucocytes. It was concluded that while the escape of bile into the blood as the result of parenchymatous disintegration could not be excluded, the histological evidence was in favour of the assumption that the jaundice was obstructive. There was no evidence that the jaundice was of haemolytic origin.

The gall bladder in the two cases in which it was examined microscopically showed in one mucous catarrh, and in the other subacute inflammation. The gall bladder was available for micro-

scopical examination in a third, subsequent, case; subacute inflammation was present.

The pancreas. Dr. Turnbull has examined the pancreas microscopically in six cases of acute obstruction of the liver by trinitrotoluene. Digestion was present in all. In three cases it was universal, being in two associated with infiltration by putrefactive bacteria. In the three other cases there were numerous focal areas of digestion. The tissues were in no case perfectly fresh when fixed, and there was no histological evidence of inflammation within the pancreatic tissue, except mucous catarrh of the duct of Wirsung in one case. The pancreatic auto-digestion may, therefore, have been a post-mortem phenomenon. Similar pancreatic digestion was found, however, in cases of acute destruction of the liver attributed to salvarsan and other poisons, and in some of these cases it undoubtedly occurred before death. It is possible, therefore, that the pancreatic digestion affords additional evidence of inflammation of the gall-bladder and common bile-duct in cases of destruction of the liver by trinitrotoluene and other poisons.

The kidneys, examined in six cases, were enlarged, rounded, and flabby. The breadth of the cortex was increased in relation to that of the medulla. Fatty degeneration was present in all, being greatest in the first and second convoluted tubules and the ascending loops of Henle, less in the glomerular endothelium and in the collecting and discharging tubules.

The myocardium, when examined, showed extensive fatty degeneration.

Haemorrhages, petechial and numerous, were present in three cases, and no records were available in the remaining four cases. The cause of the haemorrhages was probably a fatty degeneration of capillary endothelium. Such a degeneration was found in the cerebral capillaries in two cases in which the *brain* was available for microscopic examination.

In the same paper is a record of the examination of three cases of severe anaemia without jaundice. In two cases a complete necropsy was made by Dr. Turnbull, and the anaemia was proved to be aplastic. A summary is given of the microscopic findings in the first of these two cases (male, aged 32). The visceral changes were those of a severe anaemia, fatty degeneration of vascular endothelium being very conspicuous. There was very little haematogenous marrow. In such as was present erythroblastic activity preponderated, and megakaryocytes were very scarce. In the marrow there were numerous plasma cells and large phagocytes. The latter contained pyknotic nuclei, erythroblasts, erythrocytes, and large granules of iron from ingested erythrocytes. Evidence of phagocytosis of erythrocytes was found also in the spleen and in the lymphatic glands, being very slight in the latter. The liver weighed 3 lb. 0 $\frac{3}{4}$ oz. The hepatic cells in the peripheral zones of the lobules were loaded with fine granules of iron. Dr. Turnbull considered that the destructive agent had acted upon the cells in the blood-forming organs, and also upon the circulatory erythrocytes. In the second case (male, aged 37) I had examined the blood during life and had diagnosed aplastic anaemia. The liver weighed 3 lb. 4 oz. The microscopic

examination of the tissues had not been made when Dr. Turnbull wrote his paper, but has been made since. The findings resembled closely those in the first case. Phagocytosis of red corpuscles in the lymphatic glands was greater, and in the spleen there were numerous cells containing coarse granules of iron. In the marrow there was abundant evidence of phagocytosis of erythrocytes, but in sections from the humerus, three ribs, the sternum, and two lumbar vertebrae, only six phagocytic cells were found to contain nucleated cells. There were fewer plasma cells in the marrow. The hepatic cells contained abundant granules of iron. In the kidney there was slight, subacute, parenchymatous nephritis.

In the third case (female, aged 38) no complete examination had been made of the blood during life, but the morphological characters of the blood were said to resemble those in the above two cases of aplastic anaemia. The marrow was not examined at the post-mortem. The liver weighed 2 lb. 5 oz. Portions of liver and kidney were received for examination. The liver contained areas of destruction similar to those in the cases of fatal jaundice, except that the fibrosis was much denser, and elastic fibrils had been formed therein. It also showed a fatty degeneration such as occurs in severe anaemia, but no iron was demonstrated by potassium ferrocyanide and warm hydrochloric acid.

Dr. Turnbull received, subsequently, tissue from three other cases of fatal anaemia in trinitrotoluene workers. In one case (male, aged 44) I had found the blood to show during life the typical changes of aplastic anaemia, and the nature of the anaemia was confirmed by examination of the bones by Dr. O'Donovan at the post-mortem examination. The liver (2 lb. 0½ oz.) contained extensive areas similar to those found in fatal cases of jaundice, but occupied by dense fibrosis; elastic fibres had not yet been found in the fibrous tissue. Central fatty degeneration due to anaemia, and an abundant deposit of iron in the hepatic columns were also present. There was phagocytosis of red corpuscles in the submaxillary lymphatic glands, and a very little iron in the spleen. The marrow sent for examination was unfortunately taken from the lower end of the femur, which would normally have been free from haematogenous elements at the age of the patient; it was lipomatous and contained no phagocytes. In a second case (female, aged 21) the aplastic nature of the anaemia was proved by examination of the bone-marrow at the necropsy. The liver (1 lb. 11 oz.) was received for microscopic examination. It contained areas of extensive lobular destruction and of portal fibrosis similar to those in fatal cases of jaundice, but occupied by dense fibrous tissue in which elastic fibrils had been formed. It also contained abundant haemosiderin.

In the third case (female, age 20) no examination had been made of the blood during life, and the post-mortem examination was incomplete. There were numerous petechial haemorrhages on the trunk and limbs. There had been haemorrhage from the vagina for eight days before death. The anaemia was ascribed to haemorrhage following abortion. The liver weighed 2 lb. 3 oz.; a portion was received for microscopic examination. This showed healed

small, trinitrotoluene lesions. It also showed a central fatty degeneration such as is found in severe anaemia, but no haemosiderosis. It is impossible to determine whether the anaemia in this case was due to trinitrotoluene, and whether it was aplastic. The severity of the anaemia and abundance of petechial haemorrhages favour aplastic anaemia rather than traumatic anaemia of eight days' duration.

Of six cases, therefore, of severe anaemia in subjects who had worked with trinitrotoluene, in four the anaemia was proved to be aplastic. In these four cases there was conspicuous haemosiderosis of the liver; in two there were also areas of previous hepatic destruction by trinitrotoluene. In both the two remaining cases there had been previous hepatic destruction, and haemosiderosis of the liver was absent. In one case the blood, examined during life, was said to resemble morphologically that of the aplastic anaemias, and the clinical course resembled closely that of the cases of aplastic anaemia. In the other the cause and nature of the anaemia are hypothetical.

The changes found in the livers of fatal jaundiced cases and the occurrence of jaundice followed by recovery has raised the important question of the permanent liver damage inflicted upon the recovered cases, and more important still the possibility that less severe but continually progressive changes are present in a considerable proportion of all T.N.T. workers.

As mentioned above, trinitrotoluene lesions were found in the livers of four of the six cases in which death was due to anaemia. In only two of these cases was the whole liver sent for examination; in both there was extensive scarring, suggesting to the naked eye the fibrosis of acquired syphilis. In one case (liver 2 lb. 0½ oz.) the patient had had an attack of toxic jaundice in April 1916, returned to the factory in August 1916 but did not handle trinitrotoluene, left the factory towards the end of December 1916, and died on February 16, 1917. In the other (liver 1 lb. 11 oz.) the patient had had an attack of toxic jaundice in September 1917, never returned to work, and died on March 26, 1918. In neither of the two cases in which portions only of the livers were received for examination was there a history of a previous attack of toxic jaundice; in one (liver 2 lb. 3 oz.) the patient had had an attack of T.N.T. gastritis on January 2, 1917, left work on March 31, 1917, and died on April 20, 1917. In all four cases the lesions were found on histological examination to have healed, and there was no evidence of progressive destruction of the liver. *Four livers of healthy T.N.T. workers instantaneously killed as the result of an explosion were examined by me and no abnormal changes were detected.* When one considers the small incidence of severe toxic jaundice and the somewhat larger and still small incidence of mild and recoverable toxic jaundice, it is possible that a number, somewhat larger still, of cases exist in which liver damage has occurred to a degree insufficient to cause symptoms. But such evidence as is available leads one to believe that any minor liver damage is recoverable and does not tend to progress.

A description of the pathological changes set up by absorption of

T.N.T. is scarcely complete without some mention of the similar action of other toxic substance.

The blood changes and liver necroses had been previously found to occur in benzol poisoning by Selling (13), Klemperer and Hirschfeld (14), and Heffter (15). The production of jaundice in animals by toluylenediamine was described by Stadelmann (16).

Blood changes, but of a different nature are commonly present among dinitrobenzene workers and were described by Malden (17). Fatal cases of toxic jaundice due to this drug are rare, but a case is described by Spilsbury (10), together with five fatal cases of 'aeroplane dope' poisoning with very similar liver changes. The poisonous substance in aeroplane dope was first shown by Heffter and Joachimoglu (18) to be tetrachlorethane. Marked destruction of the liver parenchyma has occurred, but to a very variable extent, in spirochaetosis ictero-haemorrhagica (19). Very similar lesions have been found in the liver in sporadic cases of acute yellow atrophy of unknown cause, and more recently in salvarsan poisoning. The lesions in the salvarsan cases are described by Dr. Turnbull (20) as almost identical with those set up by T.N.T. In phosphorus poisoning fatty degeneration is a more conspicuous feature.

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